THYMUS CELL MATURATION

Studies on the Origin of Cortisone-Resistant Thymic Lymphocytes*

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It is well known that various glucocorticoids have a profound effect on lymphoid tissues and immune responses (1, 2). Nowhere is this more evident than in the thymus, where the lymphocytolytic effects of cortisone occur within hours, and lead to a dose-dependent organ weight loss. However, not all lymphocytes in the thymus are lysed by the action of cortisone. The cortisone-resistant subpopulation found mainly in the medullary areas of the thymus consists of cells lacking the thymus-specific alloantigen TL (3); these cells account for most of the graft-vs.-host immune reactivity of the thymus (4–6).

Previous studies have indicated that large thymic lymphocytes divide and differentiate to small lymphocytes, at least some of which migrate to peripheral lymphoid sites (7). The purpose of this study was to investigate whether some of these dividing cortical cells acquire cortisone resistance as a function of differentiation, or whether the cortisone-resistant cells exist as a distinct and self-renewing population. We have used the technique of labeling of surface thymus blast cells (8) followed by a cold thymidine chase to investigate the origin of cortisone-resistant cells in the thymus.

Materials and Methods

Each 5-day-old BALB/c mouse was anesthetized on ice, its sternum split and retracted, and the ventral surface of both thymus lobes exposed carefully by dissection of the overlying tissues. At this point each mouse received 0.02 ml of a 1×10^{-2} M solution of "cold" thymidine intraperitoneally. (This is equivalent to approximately 10^{-5} M when distributed throughout the total body water, and was chosen as a dose which prevents reutilization of [^{3}H]thymidine at the nucleoside level. Of course, absorption and distribution of the cold thymidine is delayed until the mouse is warmed up after surgery and regains cardiovascular and respiratory function.)

0.5 μl (0.5 μCi) of [^{3}H]thymidine deoxyriboside (NEN 620-041, 6.7 Ci/mM, 1 mCi/ml) was expressed into a droplet from a microsyringe and touched to the surface of one thymus lobe. The same process was repeated for the other thymus lobe. Non-surface-associated fluids were

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absorbed with a cotton-tipped applicator by daubing the area superior and inferior to the thymic lobes. Usually 15–30% of the applied tritium was recovered on the cotton-tipped applicator. The mice were then sutured, warmed, and returned to their mothers. They received daily subcutaneous injections of the initial dose of cold thymidine.

RESULTS

This technique regularly labels the outer cortical large lymphocyte layer, four to eight cells deep in these (5-day-old) mice. Fig. 1 A demonstrates this

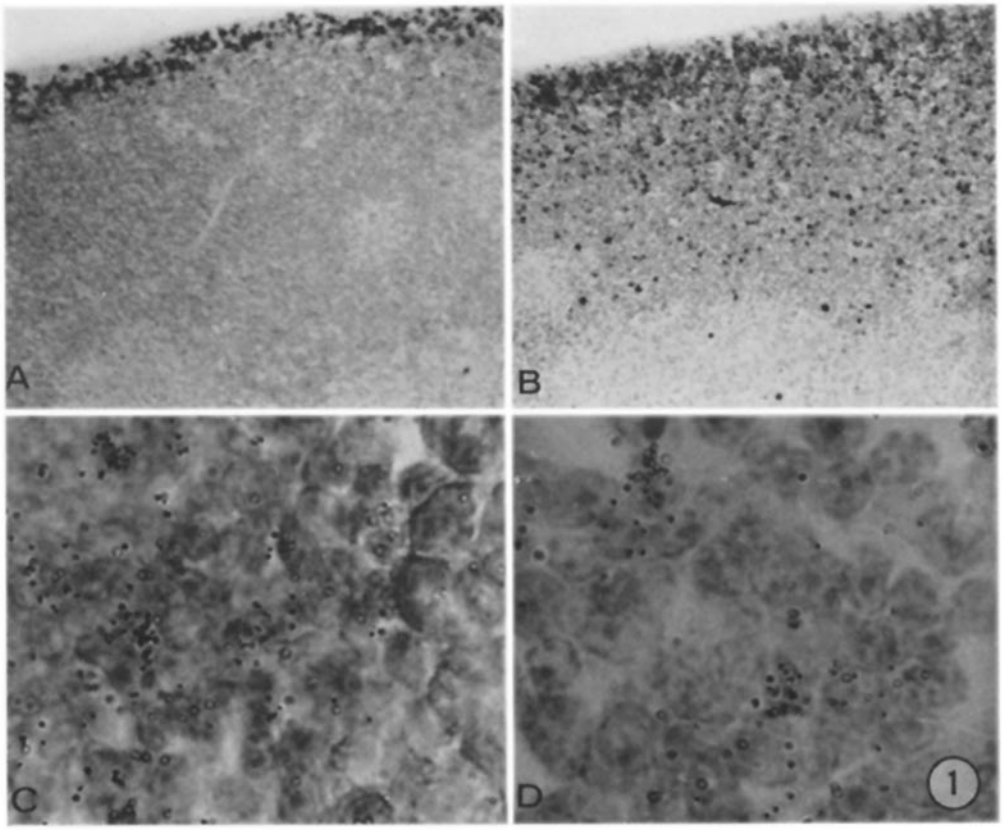

FIG. 1. Autoradiographic evidence of intrathymic cellular migration after transcapsular administration of tritiated thymidine. The times noted are the labeling to sacrifice intervals. (A) Low-power view (× 154) of thymus 3 h after labeling. The black dots represent heavily labeled cells (3 mo exposure). (B) Low-power view (× 154) of thymus 24 h after labeling (3 mo exposure). (C) High-power view (× 1,540) of thymus cortex 48 h after labeling. The outer cortex blast cells are on the extreme right of the photograph, and the heavily labeled mid-cortical cells are to the left (1 mo exposure). (D) High-power view (× 1,540) of thymic medulla 96 h after labeling. Two highly labeled cells (>10 grains) and numerous lightly labeled cells are seen (1 mo exposure). All tissues were fixed in formal alcohol and stained with methyl green-pyronin.

labeling pattern 3 h after surface labeling. By 24 h (Fig. 1 B) heavily labeled cells are are found penetrating the midcortical regions, with some cells reaching the juxtamedullary region. By 2 days after surface labeling the outer cortical large lymphocytes have much reduced grain counts (Fig. 1 C) presumably either via dilution by division, or perhaps via replacement. After 24 h many labeled cells appear in the medulla (Fig. 1 D). Thus, there is a progression of cells from outer cortex to medulla. This technique does not allow for any estimation of a "reverse flow" of cells from medulla to cortex, and therefore multiple patterns of cell migration cannot be excluded. Nor is this technique quantitative, in that the acid-precipitable ^3H counts vary by as much as 100% from mouse to mouse. Therefore, we cannot determine what proportion of medullary lymphocytes are descendants of cortical precursors, or what proportion of cortical lymphocytes eventually appear in the medulla.

If the outer cortical lymphocytes are cortisone sensitive, one can test whether their descendants gain cortisone resistance as a function of maturation and/or migration.

Accordingly, thymus-labeled mice were injected intramuscularly with 2.5 mg hydrocortisone acetate (HC)[1] either at the time of labeling or 1, 2, or 3 days later. All hosts were sacrificed 24 h after hydrocortisone injection.

Administration of HC to these hosts resulted in a striking degree of cortical thymocyte pycnosis by 24 h, with only minimal medullary thymocyte pycnosis (Fig. 2 A; references 9, 10). If one examines the thymus of a host labeled and injected with HC the same day, one sees that the only labeled cells present are in the cortex, and that virtually all label is over pycnotic nuclei (Fig. 2 B). If, however, one delays HC injection until 2–3 days after labeling, almost all medullary label is over intact cells, whereas all cortical label is over pycnotic cells (Figs. 2 C, D; Table I). Thus, it appears that at least some cortisone-resistant thymus cells are derived from cortisone-sensitive precursors.

Since it was possible that the labeled, HC-resistant, medullary thymocytes were derived from the small (0.2%) proportion of cortical cells which are HC resistant, we tested the intrathymic migration potential of HC-resistant cortical cells.

5-day-old BALB/c mice were labeled as before, and divided into three groups: those injected with HC at the time of labeling (group 1), those injected with HC on day 3 after labeling (group 2), and uninjected controls. All hosts were sacrificed on day 4 for analysis.

If HC-sensitive cortical cells were the precursors of HC-resistant medullary cells, one should not see labeled medullary cells in the first group, but should in the second. On the other hand, if HC-resistant medullary cells were derived from HC-resistant cortical cells, one would not expect to see a differential effect of

[1] *Abbreviation used in this paper:* HC, hydrocortisone acetate.

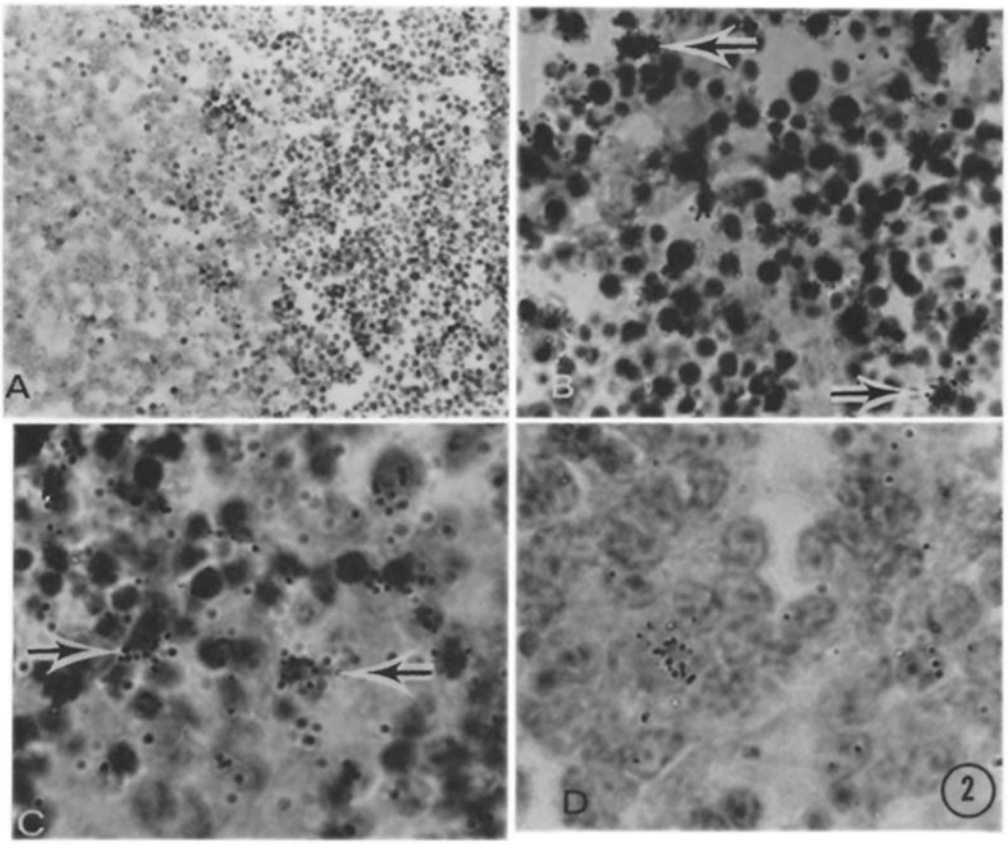

FIG. 2. Autoradiographic evidence of cortisone-sensitive (pycnotic) and cortisone-resistant cells among outer thymic cortical lymphocytes and their descendants. (A) Low-power view (× 385) of thymus 24 h after administration of HC and surface labeling. To the right are mostly pycnotic cortical thymocytes, and to the left are predominantly intact medullary thymocytes. (B) High-power view (× 1,540) of thymus cortex shown in A; arrows denote labeled (pycnotic) cells. (C) High-power view (× 1,540) of thymus cortex 48 h after surface labeling and 24 h after HC administration; arrows denote labeled (pycnotic) cells. (D) High-power view (× 1,540) of medulla from thymus shown in C. A single, highly labeled (>10 grains), intact cell is seen surrounded by unlabeled and lightly labeled cells. Methyl green-pyronin.

the time of HC administration on the incidence of labeled medullary cells by day 4. The results are presented in Table II. HC administered on the day of labeling prevents the appearance of labeled cells in the medulla by day 4, whereas HC on day 3 does not.

Are all medullary lymphoid cells derived from cortical ancestors, or is the medulla a potential traffic zone, as suggested by Brumby and Metcalf (11)?

Adult BALB/c hosts were injected with HC (5 mg) the day before or the day of intravenous injection of 1×10^8 [^{3}H]uridine-labeled spleen and lymph node cells. All hosts were sacrificed on the day after cell injection, and the spleen, thymus, and brachial node were removed for analysis.

In all three groups (Table III), the nodes and spleen were the site of extensive homing, whereas the thymic medulla was devoid of labeled cells. We could not reveal any thymic traffic zones in untreated or HC-injected hosts, and therefore propose that medullary cells are of recent thymic origin.

DISCUSSION

We interpret these experiments as demonstrating the maturation of thymic, medullary, HC-resistant cells from cortical HC-sensitive precursors, and not from an HC-resistant, self-renewing subpopulation within or without the thymus. Whether this is an actual maturation event or an anatomical limitation

TABLE I

Comparison of Cortisone Sensitivity of Descendants of Labeled Thymic Outer Cortical Cells

Labeling to sacrifice interval	Site of cells counted	Labeled cells	
		No. intact	No. pycnotic
h			
24	Cortex	2	998
24 (no HC)	Cortex	99	1
96	Medulla	96	4

All hosts labeled at 5 days of age. 2.5 mg HC was given intramuscularly to different groups of mice at 5 or 8 days of age, followed in 24 h by sacrifice for analysis. Only highly labeled (>6 grains) cells were counted. Pycnotic cells were identified as homogeneous staining blue or pink nuclei without evidence of a cytoplasmic rim. Intact cells were identified by the presence of reticulated nuclei with both euchromatic and heterochromatic regions, and a thin cytoplasmic rim. The two intact cells in the 24 h HC cortex were in the midcortex, were larger than the surrounding pycnotic cells, and possessed abundant pyroninophilic cytoplasm. It is likely that they represent labeled epithelial cells.

TABLE II

*Failure of Cortisone-Resistant Thymic Cortical Cells to Migrate to Medulla**

Labeling to sacrifice interval	HC to sacrifice interval	Thymic region analyzed	Cells with grain counts of:			
			0–3	4–6	7–10	>10
days	*days*					
4	4	Cortex‡	832	36	24	108
		Medulla	997	2	1	0
4	1	Medulla	920	62	10	8

* All hosts labeled with transcapsular technique at 5 days of age. HC was given intramuscularly to group 1 on the day of labeling, and to group 2, 3 days later. All hosts were sacrificed 4 days after transcapsular labeling.

‡ All labeled cells by this time were nonlymphoid.

TABLE III

*Absence of Influx of Labeled Lymphoid Cells to Thymic Medulla of HC-Treated and Untreated Mice**

Day HC injected	Day cells injected	Organ examined	Labeled cells (>6 grains)	Unlabeled cells (<6 grains)
1	0	Thymic medulla	0	500
		Spleen	280	220
		Brachial node	226	274
0	0	Thymic medulla	0	500
		Spleen	279	221
		Brachial node	186	314
—	0	Thymic medulla	0	500
		Spleen	208	292
		Brachial node	212	288

* All mice were injected intravenously with 1×10^8 syngeneic spleen and lymph node cells which had been labeled in vitro with 10 μCi/ml of [^{3}H]uridine for 60 min, washed, and resuspended in 1×10^{-4} M [^{1}H]uridine before injection. All hosts were sacriced 24 h after cell injection.

of biologically active HC to cortical cells has not been settled by this study. In vitro analysis of cellular sensitivity to HC of the two cell populations, or of cytoplasmic soluble receptors specific for 11-β-glucocorticoids (12–14) might distinguish between these possibilities.

Since thymus cell migrants to thymus-dependent areas of lymph nodes and spleen do not appear in these hosts until labeled cells have reached the thymic medulla, it seems likely (although not proven) that migration is a late event in maturation, and that emigration occurs from medullary sites. We have now apparently revealed another late event in thymocyte maturation, the acquisition of resistance (or perhaps more properly the loss of sensitivity) to the lytic effects of HC. Yet another presumed (though not proven) late event is the quantitative loss of TL antigens (3). It is therefore of considerable interest that all of the acquired immune functional characteristics of thymus cells may be ascribed to the cortisone-resistant and TL-negative cells in the thymus (15). It is possible that the acquisition of immune competence and loss of HC sensitivity by putative thymus cell migrants are not unrelated events. Perhaps only "properly" matured thymus cells lose glucocorticoid sensitivity, and thereby are assured survival in an environment which may contain relatively high concentrations of glucocorticoids on an episodic basis (16).

SUMMARY

Outer thymic cortical large lymphocytes were labeled by transcapsular administration of tritiated thymidine. By 2–4 days after labeling, small and medium labeled lymphocyte descendants were found throughout the cortex and in the medulla. The labeled cortical lymphocytes undergo pycnosis after parenteral administration of hydrocortisone 24 h previously, but their (labeled)

medullary descendants do not. In addition, parenteral administration of hydrocortisone at the time of surface labeling results in the absence of the appearance of labeled medullary descendants.

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REFERENCES

1. Dougherty, T. F. 1952. Effect of hormones on lymphatic tissue. *Physiol. Rev.* **32**:379.
2. White, A., and A. L. Goldstein. 1972. Hormonal regulation of host immunity. *In* Immunogenicity. F. Borke, editor. North-Holland Publishing Co., Amsterdam. 334.
3. Schlesinger, M., and V. K. Golokai. 1967. Loss of thymus-distinctive serological characteristics in mice under certain conditions. *Science (Wash. D.C.).* **155:** 1114.
4. Cohen, J. J., M. Fischbach, and H. N. Claman. 1970. Hydrocortisone resistance of graft vs. host activity in mouse thymus, spleen, and bone marrow. *J. Immunol.* **105:**1146.
5. Blomgren, H., and B. Andersson. 1969. Evidence for a small pool of immunocompetent cells in the mouse thymus. *Exp. Cell Res.* **57:**185.
6. Blomgren, H., and B. Andersson. 1970. Characteristics of the immunocompetent cells in the mouse thymus: cell population changes during cortisone induced atrophy and subsequent regeneration. *Cell. Immunol.* **1:**545.
7. Weissman, I. L. 1967. Thymus cell migration. *J. Exp. Med.* **126:**291.
8. Bryant, B. J. 1971. Quantitative studies of thymocytokinetics. *In* Morphological and Fundamental Aspects of Immunity. K. Lindahl-Kiessling, G. Alm, and M. Hanna, editors. Plenum Publishing Corporation, New York. 103–112.
9. Ishidate, M., and D. Metcalf. 1963. The pattern of lymphopoiesis in the mouse thymus after cortisone administration or adrenalectomy. *Aust. J. Exp. Biol. Med. Sci.* **41:**637.
10. Kaplan, H. S., C. S. Nagareda, and M. B. Brown. 1954. Endocrine factors and radiation-induced lymphoid tumors of mice. *Recent Prog. Horm. Res.* **10:**293.
11. Brumby, M., and D. Metcalf. 1967. Migration of cells to the thymus demonstrated by parabiosis. *Proc. Soc. Exp. Biol. Med.* **124:**99–103.
12. Baxter, J. D., A. W. Harris, G. M. Tomkins, and M. Cohn. 1971. Glucocorticoid receptors in lymphoma cells in culture: relationship to glucocorticoid killing activity. *Science (Wash. D.C.).* **171:**189.
13. Kirkpatrick, A. F., R. J. Milholland, and F. Rosen. 1971. Stereospecific glucocorticoid binding to subcellular fractions of the sensitive and resistant lymphosarcoma P1798. *Nat. New Biol.* **232:**216.
14. Hackney, J. F., S. R. Gross, L. Aronow, and W. B. Pratt. 1970. Specific glucocorticoid-binding macromolecules from mouse fibroblasts growing *in vitro*. *Mol. Pharmacol.* **6:**500.
15. Leckband, E., and E. A. Boyse. 1971. Immunocompetent cells among mouse thymocytes: minor population. *Science (Wash. D.C.).* **172:**1258.
16. Selye, H. 1946. The general adaption syndrome and the diseases of adaptation. *J. Clin. Endocrinol. Metab.* **6:**117.