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Selective Depletion of Thymus Dependent Areas in Lymphoid Tissue by Tilorone[1] (38079)

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The thymus is partly responsible for the development of peripheral lymphocyte populations in fetal and infant mammals. As a result, animals that have congenital absence of the thymus (1), or have been subjected to neonatal thymectomy (2, 3), or treatment with antithymocyte serum (4, 5) have few lymphocytes in certain areas of spleen, lymph nodes, and Peyer's patches. These areas are referred to as "thymus dependent." In the present work, we show that a chemical agent, tilorone, causes a rapid, transient, selective depletion of these same areas in adult animals.

Methods. Tilorone-(2,7-bis-(diethylaminoethoxy) fluoren 9-one) hydrochloride was dissolved freshly in saline. It was administered subcutaneously or by gastric tube to young adult male or female, Lewis or Sprague–Dawley derived rats, or Swiss/ICR mice. Control animals were given saline. Surgical procedures were done under ether anesthesia and wounds were closed with silk sutures and metal clips. Adrenalectomized rats were maintained on saline as sole fluid. The animals were killed by exsanguination under anesthesia. Tissues were fixed in Bouin's fluid or 10% formalin, embedded in paraffin, sectioned and stained with hematoxylin–eosin.

Results. Spleens taken 24 hr after a single subcutaneous dose of 25, 50, or 100 mg/kg of tilorone, or after 100 or 200 mg/kg per os revealed depletion of small lymphocytes around most of the central arterioles in the white pulp (Fig. 1). Germinal centers and red pulp had no abnormalities; perifollicular sheaths sometimes had minimal shrinkage. Lymph nodes had a striking depletion of lymphocytes from the paracortical lymphoid masses ("diffuse cortex," "mid and deep cortex") (Fig. 2). Cortical nodules, germinal centers, and medulla appeared normal. Peyer's patches in the small intestines had distinct depletion of lymphocytes in the "corridors" between the nodules; the latter were intact.

The areas of depletion described above are exactly those known to be thymus-dependent from studies of thymectomy and thymic aplasia (1–5). Nevertheless, the thymus itself had only minor changes (a few phagocytes with cellular debris), not distinguishable from controls. It is interesting that antilymphocyte serum also depletes thymic-dependent areas of spleen and lymph nodes while leaving the thymus intact under some conditions (4).

Depletion of paracortical areas was seen regularly in mesenteric nodes. The same change was detected in mediastinal, inguinal, axillary, elbow, and lumbar nodes, but not as regularly, perhaps because of their small size. Depletion was detected only rarely in cervical nodes most of which were large and had an abundance of plasma cells, indicating strong prior antigenic stimulation. The distribution and intensity of lymph node changes were the same after oral, subcutaneous, and intraperitoneal administration of tilorone, and the depletion was not

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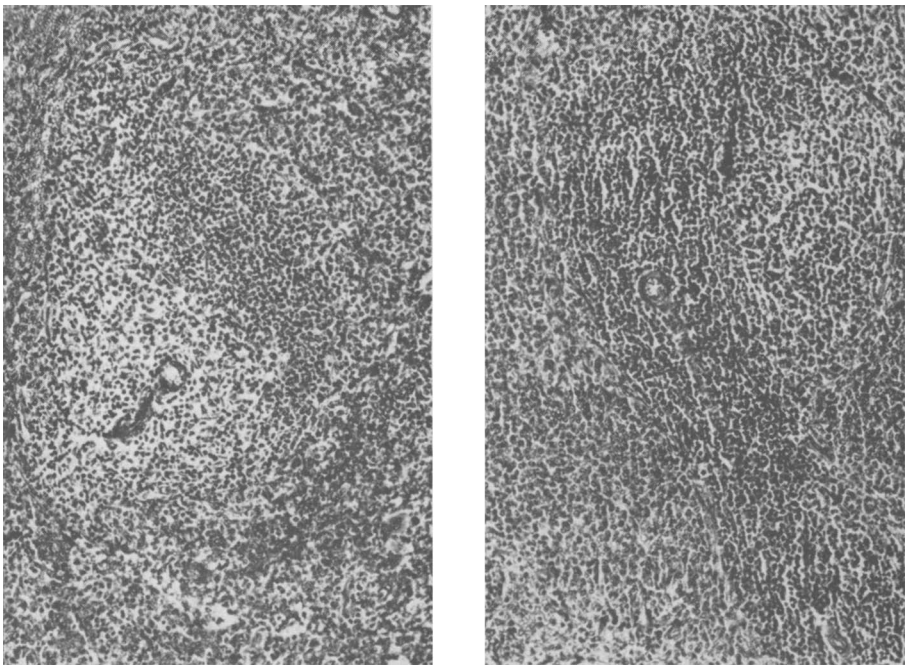

FIG. 1. After tilorone treatment (left frame) there are very few cells around the central arteriole of the splenic white pulp (near center), whereas the same area in the control (right frame) is densely populated with small lymphocytes. The areas of intermediate population density (above and right of central arterioles) are germinal centers, unaffected by tilorone. Hematoxylin–eosin stain (×205).

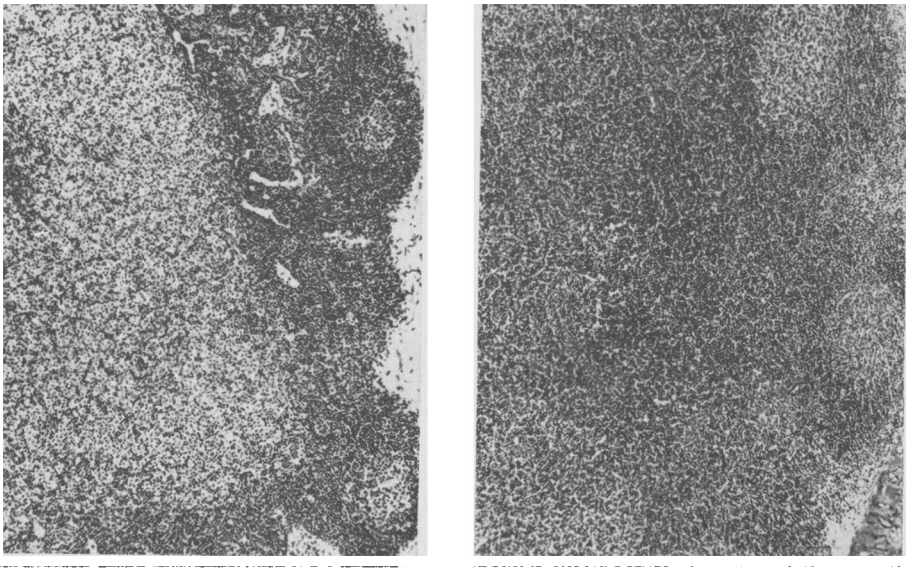

FIG. 2. After tilorone treatment (left frame), there is severe depopulation of the paracortical lymphoid mass of mesenteric lymph node, compared to the control (right frame). Cortical nodules with germinal centers are not affected by the treatment (right margin of both frames). Hematoxylin–eosin stain (×70).

accentuated in regional nodes draining sites of subcutaneous inoculation.

The changes in spleen, lymph nodes, and Peyer's patches were not yet developed 8–12 hr after sc or oral treatment of rats with tilorone, and were already decreased markedly by the second day and absent by the third day after treatment. Even at 24 hr, when the depletion was fully developed in lymph nodes, lymphocytes were present in and immediately around the walls of the postcapillary venules of the paracortical lymphoid masses, suggesting that repopulation was already under way. One dose of tilorone also produced a leukopenia which was due to an absolute lymphopenia and was associated with an absolute neutrophilia. These effects also reached their peak within 24 hr and reverted to normal by 2 or 3 days.

The effects of tilorone on lymphoid tissue have been observed consistently in over 20 experiments on adult rats and mice in our two laboratories. Adolescent (6 wk old) rats had equally obvious changes, but weanlings (4 wk old) had distinctly less depletion of T-cell areas. The depletion was fully in evidence in adult rats whose adrenals were removed 3 days before tilorone treatment. Therefore, the phenomenon cannot be caused by adrenal stimulation with liberation of corticosteroids. Furthermore, the thymus did not show stigmata of steroid excess, and the selective changes in peripheral lymphoid organs induced by tilorone were not duplicated by intraperitoneal injection of steroids (dexamethasone, 5, 0.5 or 0.05 mg/kg once, or 0.5 mg/kg on 2 consecutive days).

Nor were the effects of tilorone dependent upon the presence of spleen or thymus. Splenectomy of three rats 1 wk before tilorone did not prevent depletion of thymic-dependent areas in lymph nodes and Peyer's patches. Thymectomy 12, 5, or 3 days before tilorone did not interfere with its effects on the peripheral lymphoid tissues (eight rats). Thymectomy alone did not cause any detectable depletion.

Complete prevention of lymphoid depletion by tilorone was obtained by prior treatment with the same dose of the drug for 1, 2, or 3 days. Rapid adaptation to the effects of the drug (tachyphylaxis) was also noted in the peripheral white blood cell counts in both mice and rats (Table I and Ref. 6).

Discussion. Cytotoxic drugs usually deplete lymphoid tissue without evidence of selectivity for thymus dependent areas. This is also true of the lymphocyte-mobilizing agent, polymethacrylic acid (7), while the possible selective effects of polysaccharide polysulfates have not been clearly demonstrated (8).

Tilorone has been under investigation for several years because of its activity against certain viruses and tumors (9–11). Depletion of lymphocytes in spleen and blood has been noted in earlier studies (6, 12) but the related changes in lymph nodes and Peyer's patches were not observed, nor was the correlation with thymic dependent areas reported. This correlation is of great interest because the drug inhibits experimental allergic encephalomyelitis, adjuvant arthritis, tuberculin skin reactions (13), all thought to be mediated by T-cells. Further work is in progress to ascertain the mechanism of the lymphoid depletion and to understand the relationship between the morphologic effects and the immunosuppressive effects on cell mediated immunity. It is of interest that tilorone enhances antibody responses to certain antigens, including one that is thought to be thymic-dependent (13, 14), an apparent paradox. It is likely that the selective ef-

TABLE I. Adaptation to Lymphopenic Effect of Tilorone.[a]

No. of daily sc injections	One day after last injection	
	White count[b]	Lymphocytes
1 Saline	7370	6380
1 Tilorone	2730	1170
2 Tilorone	5370	3360
3 Tilorone	4950	4090
4 Tilorone	6640	5660

[a] 100 mg/kg to male 2 mo old Lewis rats, 220–290 g wt.

[b] White blood cells/mm^3 in aortic blood; average of duplicate Coulter counts in each of two rats.

fects of this chemical agent will be useful for the delineation of T-cell functions and possibly for the control of T-cell hyperfunction or neoplasia.

The nature of the cells that rapidly repopulate the depleted lymphoid tissues, and the reason for their resistance to further doses of tilorone (tachyphylaxis), are under investigation. Current results suggest that part of the new population must be T-cells, as they are susceptible to lysis by antilymphocyte serum.

Summary. The chemical agent, tilorone, depleted the lymphocyte population in certain areas of the spleen, lymph nodes, and Peyer's patches. The pattern was like that caused by neonatal thymectomy, thymic aplasia, or antilymphocyte serum. The thymus itself was not affected. The selective depletion of the thymus-dependent areas was unlike the nonspecific effects of other immunosuppressive chemicals and may be related to the ability of tilorone to inhibit cell-mediated immunity selectively. The effects of tilorone were not dependent on the presence of adrenal, thymus or spleen. The depleted tissues were rapidly repopulated, and the replenished thymic-dependent areas were resistant to further doses of the same drug.

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