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The Influence of Prednisolone and Thymectomy on the Thoracic Duct Lymphocyte Population of the Rat*

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Summary

The number of mononuclear cells in the thoracic duct of 8-10 week-old rats were counted three and seventeen hours after a single dose of prednisolone and the concomitant involution of the lymphoid tissues was studied histologically.

Both in animals thymectomized at weaning age and in shamoperated controls the number of cells per volume and per hour were strongly depressed after three hours. At seventeen hours after injection the original level was reached again. The sensitivity of thoracic duct lymphocytes to prednisolone in vitro was tested, and cells from thymectomized animals were found to be about as sensitive as cells from shamoperated animals. The mechanism for the disappearance and rapid regeneration of the lymphocytes in the circulating pool is discussed.

Histologically no differences was observed between shamoperated and thymectomized animals in

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sensitivity of spleen, lymph nodes and Peyers patches. The thymus dependant areas did not show any significant steroid sensitivity as judged by lymphocytolysis.

The steroid sensitivity of small lymphocytes in various lymphoid tissue has been known for some time (1). The varying sensitivity of different tissues is also well known (2, 3). Thus the lymphocytes of the thymic cortex and in germinal centers are much more sensitive than the cells in the thymic medulla or most of the cells in lymph nodes and in the spleen white pulp. Investigation with isotope labelling of the short-lived and long-lived lymphocyte populations (4) indicate that short-lived small lymphocytes are more susceptible to the destructive effects of cortisone than are the long-lived. *Miller and Cole's* (5) studies indicate that long-lived lymphocytes are especially resistant to prednisone and cyclophosphamid.

During the last decade, numerous investigations on the role of the thymus in the development of the peripheral lymphoid tissue and the immune response have shown among other things that there is a thymus dependent population of small lymphocytes in the lymphoid tissues and circulation in the peripheral lymph and blood (6). At least a part of this population seems to be long-lived, and a memory function in immune response has been ascribed to it (7, 8).

The thymus cells in situ and the thymus dependant cells in the periphery can be identified in mice by the Θ-antigen (9). In the thymus, Θ-positive cells disappear in the course of steroid induced involution (10) but *Blomgren and Andersson* (11) have shown that immunocompetent cells remain in the thymus after such an involution. The morphological identification of these cells is still missing. Very little is known on the effect of steroid on the peripheral immunocompetent T-cells population (T2 cells according to *Cantor* 12), but *Moorhead and Claman* (13) observed the appearance of cells sensitive to anti Θ-serum in the bone marrow after steroid treatment.

The modern views on cell cooperations in immune response warrant more penetrating studies of the corticosteroid sensitive lymphocyte population which is the dominating cell type in the thymic cortex and in the germinal centers of the peripheral lymphoid tissues.

The aim of our present investigations is to attempt to define the steroid sensitive lymphocyte population, and to correlate the steroid sensitivity with life span and thymus dependence. In this paper the steroid sensitivity of the thoracic duct lymphocytes and lymphoid tissues in rats thymectomized at weaning age is compared to that of non-thymectomized controls.

Material and Methods

Specific pathogen free rats of the Buffalo strain were used. They were thymectomized at the weaning age of three weeks, and were used for the experiment at the age of about 8-10 weeks and at a body weight of about 300-350 gm.

As corticosteroid preparation a prednisolone sodium succinate (Schering Meticortelone Soluble) was used. The rats were injected intramuscularly in the thigh, and the dosage used in most experiments was 8 mg per 100 g body weight. In a few experiments 16 mg per 100 g body weight was used. The drainage of the thoracic duct was started 2-3 or 17-18 hours after prednisolone injection. The open neck technique of *Rhine-*

hart (14) was used. The animals were anaesthetized with nembutal. The drainage time was usually about 60 minutes but was never less than 30 minutes. About 15 minutes after the start of the drainage a capillary blood sample was taken from the tail and the number of blood mononuclear cells was counted.

At the end of the drainage period the animals were killed by opening the chest, and the spleen and lymph nodes (thoracic, axillary, lumbar and mesenteric) and adrenal glands were dissected out, weighed and fixed in formalin. The number of lymphocytes in the thoracic duct lymph was counted in a haemocytometer, and the number of cells per hour was calculated.

In vitro methods: Thoracic duct cells from 2 or 3 non-prednisolone treated thymectomized animals were pooled as were cells from a like number of shamoperated animals. In plastic tubes 1.5 million cells were incubated in Eagles MEM with 1% of crystalline bovine serum albumin. The tubes were gassed with 5% carbon dioxide in air, closed and incubated for 18 hours at 37°C. The method is essentially that described by *Burton* et al. (15). Before incubation the total number of cells was counted in a haemocytometer, and at the end of the experiment the numbers of morphologically intact and pycnotic cells were counted.

Results

The number of animals used, their body weight and organ weights are shown in Table 1. No significant decrease in body weight, and no increase in the weight of the adrenals were observed in the thymectomized animals compared to sham-operated controls. The spleens were not reduced in the thymectomized rats compared to the sham-operated ones. The lymph nodes were smaller in the groups of thymectomized rats but the differences were not significant.

Table 1

	Rats	Body weight	Organ weights mg			
		g	Spleen	Thymus	Lymph node	Adrenals
Shamoperated, untreated	7	334 ± 8	548 ± 3	404 ± 8	239 ± 10	33.8 ± 1.9
–"–, 2-3 hours after steroidtreatment	5	323 ± 8	632 ± 41	386 ± 22	223 ± 17	35.5 ± 1.7
–"–, 17-16 hours after steroidtreatment	4	320 ± 14	537 ± 9	345 ± 43	178 ± 2	35.7 ± 2.3
Thymectomized, untreated	8	333 ± 12	610 ± 7	–	173 ± 43	34.3 ± 0.7
–"–, 2-3 hours after steroidtreatment	5	340 ± 9	600 ± 44	–	200 ± 10	35.5 ± 1.9
–"–, 17-18 hours after steroidtreatment	4	321 ± 21	666 ± 84	–	158 ± 13	36.7 ± 2.6

Compared to the sham-operated non-treated animals, the lymph nodes and spleen of the thymectomized animals showed smaller follicles with small germinal centres. No significant depletion, however, of the small lymphocytes in the so called thymus dependant areas (8) in spleen, lymph nodes of Peyers patches could be seen.

In the sham-operated animals extensive lysis of the small lymphocytes in the thymic cortex was seen 2-3 hours after prednisolone treatment but the basic structure was still

intact. In the lymph nodes a pronounced cytolysis was found in the germinal centres. In the paracortical areas, the lymphocytolysis was insignificant. The effect of the steroid treatment in the thymectomized animals was essentially the same as in the sham-operated.

In the spleen of the sham-operated rats there was a pronounced lymphocytolysis in the follicles, especially in the germinal centers; and an insignificant cytolysis among the small lymphocytes in the perivascular sheaths. The perifollicular zones were essentially preserved with larger lymphocytes or lymphoblasts, and showed only a few pycnotic cells. In the spleen of the thymectomized animals the picture was essentially the same as in the sham-operated ones.

By 17 to 18 hours later the thymus of the sham-operated animals showed a pronounced reduction of the cortex, with an almost total disappearance of the small lymphocytes. In the lymph nodes the follicles were reduced in size with small germinal centres, but pycnotic cells could still be observed. The picture was about the same in both thymectomized and sham-operated animals. In the spleen, too, the follicles were reduced with small germinal centers and a thin perifollicular zone. Fairly few pycnotic cells could be observed, most of them in the cytoplasm of macrophages in the germinal centers. The picture in the spleen of the thymectomized animals was identical with that of the sham-operated ones.

The number of cells in the thoracic duct lymph is seen in Fig. 1 and 2. 2-3 hours after steroid treatment the cells in the sham-operated animals dropped from about 40,000/μl to about 14,000 on the average. There was a significant rise in the cell-number 12-13 hours later, but the original level was not fully regained. In the thymectomized rats the untreated animals showed on the average 17,000 cells/μl which, after steroid treatment, dropped to about 7,000. The original level was regained 12 to 13 hours later.

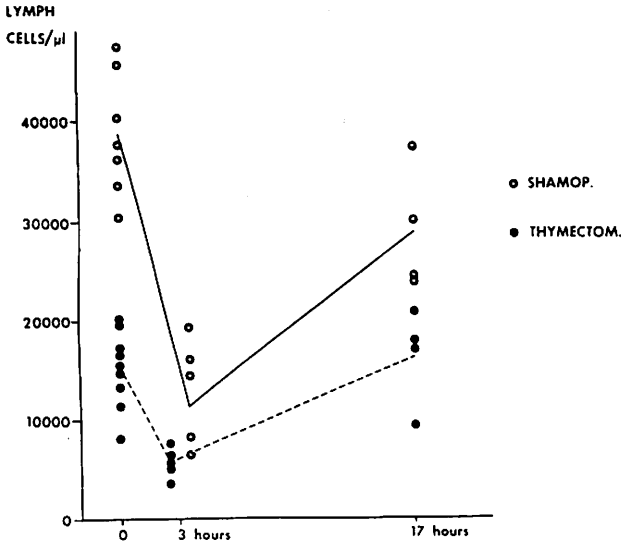

Fig. 1. The number of cells per μl in thoracic duct lymph 3 and 17 hours after a single dose of prednisolone sodium succinate.

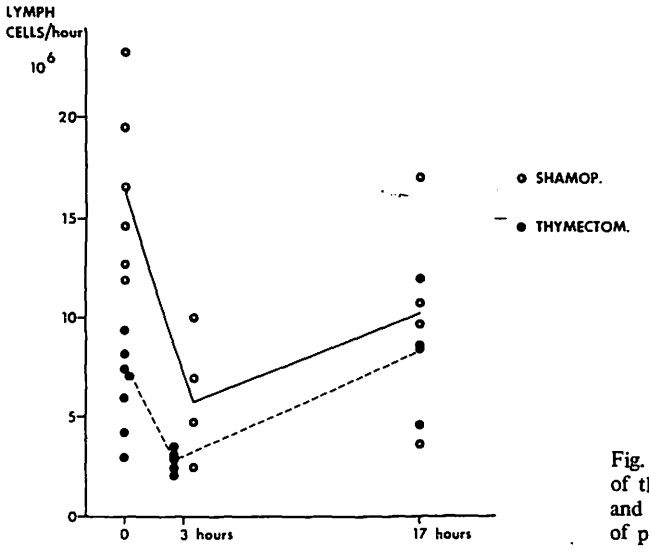

Fig. 2. The total output per hour of thoracic duct lymphocytes 3 and 17 hours after a single dose of prednisolone sodium succinate.

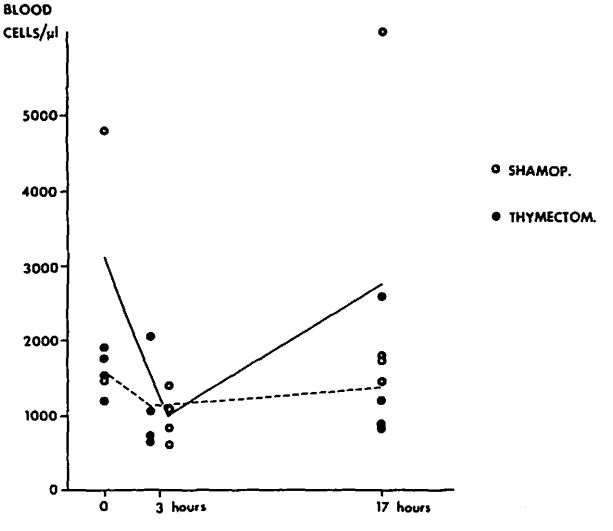

Fig. 3. The number of mononuclear cells per µl in peripheral blood 3 and 17 hours after a single dose of prednisolone sodium succinate.

The changes in the number of lymphocytes in peripheral blood are seen in Fig. 3. The effect of the steroid treatment is, in general, the same as in the thoracic duct lymph. The number of observations at same points, however, are few, and no definite conclusions can be drawn.

The results of one *in vitro* experiment is seen in Fig. 4. The maximal effect seems to be achieved with about 0.1 to 1.0 µg per ml, and with this and higher dosages there is a definite difference between the sensitivity of cells from thymectomized animals compared to cells from sham-operated animals. The former cells were less sensitive to the steroid treatment.

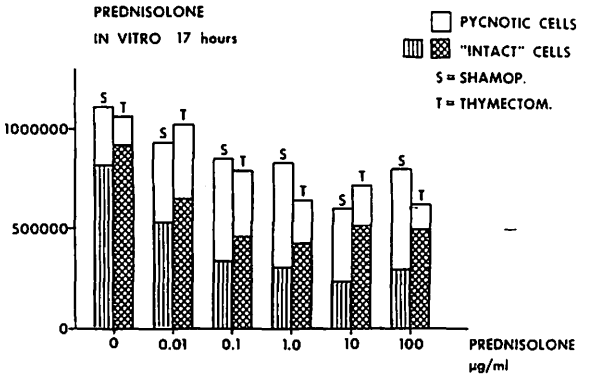

Fig. 4. The number of intact and pycnotic cells remaining after 17 hours incubation in vitro of 1.5 million cells with various concentration of prednisolone sodium succinate.

Discussion

The morphological observations did not reveal any significant difference in the steroid-sensitivity between sham-operated and thymectomized animals. It is interesting that the so called thymus-dependant areas did not show any apparent steroid sensitivity during acute involution. This may imply that the peripheral thymus-derived or thymus dependant cell population is not sensitive to the lytic action of corticosteroids, as is not sensitive to the acute effect of irradiation (16). This finding has to be confirmed in neonatally thymectomized animals.

Thymectomy of young rodents results in pronounced decrease in the output of lymphocytes in the thoracic duct. The magnitude of the response is related to the age of the animal at the time of the removal of the thymus. In young adult animals a pronounced decrease in the output of cells is observed, while most of the other alterations found after neonatal thymectomy are lacking (17, 18). The results presented here confirm these earlier observations.

After prednisolone treatment a rapid decrease in the number of thoracic duct lymphocytes is observed both in sham-operated and thymectomized animals. The decrease is most apparent in the first group but of comparable relative magnitude in the thymectomized rats. Thus, in both sham-operated and thymectomized rats, about 60 per cent of the cells disappear under the influence of steroids.

Earlier work (19) has shown that about 80 per cent of the cells in the thoracic duct are long-lived, and *Esteban*'s (4) studies have indicated that the steroid sensitive cells are more or less identical with the short-lived cells. Our findings are hardly consistent with these earlier statements, but need some other explanation. One explanation could be that no definite correlation exists between the life span of the cells and steroid sensitivity. Thus, steroid sensitive cells may be either short-lived or long-lived.

Another explanation may be that the disappearance of the lymphocytes after steroid treatment not only depends on a lysis of cells circulating in the thoracic duct, but also on a redistribution of the cells to other tissues. The rapid restitution of the number of cells after the steroid treatment may favour this view, but these findings do not exclude a mobilization of "resting" cells from some depot, or release of cells, newly formed in the lymphoid tissue.

Table 2

	Thoracic Duct. Lymph		Blood
	cells/ul 10^3	cells/hour 10^6	cells/μl
Shamoperated, untreated	38.49 ± 2.03	16.41 ± 1.62	3125 ± 1184
Shamop., 2-3 hours after steroidtreatment	12.91 ± 2.14	6.57 ± 1.23	975 ± 149
Shamop., 17-18 hours after steroidtreatment	28.85 ± 2.73	10.34 ± 2.39	2781 ± 975
Thymectomized, untreated	15.96 ± 0.98	7.63 ± 1.34	1578 ± 127
Thymect., 2-3 hours after steroidtreatment	5.79 ± 0.60	2.75 ± 0.23	1119 ± 279
Thymect., 17-18 hours after steroidtreatment	16.13 ± 2.11	8.42 ± 1.37	1375 ± 361

The works by *Moorhead and Claman* (13) and *Cohen* (20) indicates such a redistribution of thymus dependent Θ-positive cells to the bone marrow after hydrocortisone treatment.

The in vitro experiments, however, show that peripheral lymphocytes both from controls and from thymectomized animals are steroid sensitive, and are lysed at moderate steroid concentrations. This indicates that the decrease of the number of lymphocyte in the thoracic duct after steroid treatment in vivo to a certain degree depends on a cell destruction.

These questions cannot be answered from data presented in this paper. Studies on neonatally thymectomized animals, where the depletion of the thymus derived or thymus dependent cells can be expected to be more severe than in these animals operated at weaning age, can add further information. A more thorough definition of the cell population before and during steroid involution and after restitution, by the aid of isotope labelling, may help to solve the problems. Such studies are in progress.

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Lymphographic Findings in a Series of 258 Patients with Tumors of the Testes

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Summary

258 patients with a tumor of the testis were examined by means of lymphography. The spread of metastases in inguinal, iliac, paralumbar, aortic, supra-clavicular and mediastinal regions in general follows the expected pattern. Special attention must be paid to groin and low iliac regions in patients who underwent previous operations (e.g. orchidopexia, herniotomy etc.). In 10% of the cases selective supplementary phlebo/cavography should yield further information about doubtful regions in the pelvis and to the right of the lumbar spine; if there is an uncertain region to the left of the lumbar spine, follow-up examinations are suggested.

Testicular tumors form one to two percent of all malignant growths in the male. Therefore it will take years to collect a series of patients of significant value, examined by means of lymphography. It seems to be of sufficient interest therefore to summarize the results of a large clinical material.

The technical part of the examination is generally performed by standard lymphography. Principally it is possible to perform a selective lymphography directly from