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The Effect of Steroids on the Circulating Lymphocyte Population

II. Studies of the thoracic duct lymphocyte population of the guinea pig after neonatal thymectomy and prednisolone treatment.

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Summary

The effect of corticosteroids on the lymphatic tissue and circulating lymphocyte pool in the guinea pig has been studied. No signs of increased lymphocytolysis was seen and only after 1–2 weeks treatment with a long acting corticosteroid was the thymic cortex reduced and thymic weight decreased.

Three hours after injecting a short acting steroid a 30–40 per cent reduction in the thoracic duct cell count was seen in both control and thymectomized animals. Restitution to pretreatment cell level was completed in 17 hours. Mononuclear cells in the blood were markedly depressed up to 40 hours.

The changes in the circulating lymphocyte pool in the resistant guinea pig seem to be in principle the same as in the sensitive rat but less pronounced.

It can be concluded that the lymphocyte level in the circulating pool is thymus dependent but restoration of the cell count after steroid treatment is independent of intact thymic function. This data supports the hypothesis of lymphocyte trapping and redistribution after a single steroid treatment.

Key-words: Lymphocyte – Thoracic duct – Lymphoid tissue – Blood – Corticosteroid – Thymectomy – Guinea pig

Introduction

Depending on the effect of corticosteroids on lymphoid tissue, and especially the lytic effect on the thymus, animals are divided into steroid resistant and steroid sensitive species. Mice and rats are regarded as sensitive animals and the guinea pig and man as resistant. A large single dose of a corticosteroid leads to pronounced lymphocytolysis in the thymic cortex (1) and reduces the thymic weight in the mice by about 90 per cent (2), while the same dose repeated daily for one week reduces the thymus in guinea pigs by only 37 per cent (3). Some of the functional changes of cell mediated immunity found in sensitive species are also observed in steroid resistant species, but large doses or prolonged treatment are required. For reviews see *Claman* (4) and *Bach* (5).

We have earlier reported the effect of a single prednisolone dose on the circulating lymphocyte pool (i.e. in thoracic duct lymph and blood) in rats (6). In that work we found that the lymphocyte level in the circulating pool is thymus dependent but restoration of the cell count after steroid induced involution is independent of intact thymic function. The major mechanism seems to be a trapping and redistribution of lymphocytes somewhere in the body, probably in the bone marrow. The lytic effect seems to be minor, at least in the circulating pool. In spite of our limited knowledge of the significance of steroid sensitivity sensitive species are usually used to study immunologic processes.

The following investigation was done to see if the steroid resistant guinea pig has a corresponding sensitive lymphocyte population and a similar response after steroid treatment.

Material and methods

To evaluate the morphological changes 4 different corticosteroid preparations were tested. Including controls 62 guinea pigs were used.

The following preparations, doses (mg/100 g B. w.) and time intervals were used:

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A. Prednisolone succinate (Precortalon Aquosum, Organon), a short acting water soluble preparation given in one dose of 10 or 20 mg. Animals were sacrified 3 or 17 hours later.

B. Hydrocortisone (Solucortef, Upjohn), a short acting water soluble preparation given over one day in doses from 4 mg (twice) to 12 mg (twice). Animals were sacrified 1, 2 or 4 days later.

C. Hydrocortisone acetate (Hydrocortal, (Organon), a crystalline suspension given in a single dose of 10, 20 or 40 mg. Animals were sacrified 12, 24 or 48 hours later.

D. Methylprednisolone acetate (Depomedron, Upjohn), a long acting suspension given either as a single dose of 10 mg or as 2 doses of 5 mg over one week. Animals were sacrified one week after the first injection. Some animals were given 4 doses of 5 mg over 2 weeks and sacrified 2 weeks after the first injection.

Within 24 hours of birth guinea pigs were bilaterally thymectomized. The operation was performed under ether anaesthesia, dissecting out both lobes using a dissection microscope. Nonthymectomized guinea pigs served as controls. Sex distribution was equal and altogether 40 animals were used for thoracic duct drainage when adult.

Prednisolone sodium succinate (Precortalon Aquosum, Organon) was given intramuscularly in the thigh in a dose of 10 mg/100 g body weight. Three, 17 or 40 hours later thoracic duct drainage was started under anaesthesia (Mebumal 60 mg/ml intraperitoneally at 6 mg/100 g body weight). The open-neck-technique of *Reinhardt* (7), was used. The minimum requirements were drainage for 20 minutes and 0.1 cc of lymph collected, but usually drainage continued for 60 minutes. The time intervals of 3, 17 and 40 hours are the same as those we earlier used in the study of the rat thoracic duct cells. The lymph was collected in heparinized glass tubes. Lymph volume was measured and a sample taken for mononuclear cell count. The total lymph flow was then calculated in cells/hour. A blood sample was taken from the lingual vein at the end of the drainage and mononuclear and polymorphonuclear cells were counted. Lymph and blood smears were also made for microscopic examination. The animals were sacrified and the spleen, lymph nodes (thymic, paraaortic, axillary and mesenterial) and adrenals were dissected out, weighed and fixed in neutral formalin for histology.

Cell count, cell flow and organ weights are expressed as the mean and the standard error of the mean and Student's t-test is used to compare the different groups.

Results

Microscopic examination of lymphoid tissues from the neonatally thymectomized animals showed a depletion of mature lymphocytes in the intermediate and deep cortical zones in lymph nodes and a tendency to lymphocyte depletion round the central arterioles in splenic follicles. After a single prednisolone dose the morphological picture did not change at any time. The number of pyknotic cells and macrophages was not increased in any lymphoid tissue in control or thymectomized animals. The other three corticosteroid preparations did not induce an increase in the number of pyknotic cells used as a measure of lymphocytolysis. The animals injected with methyl-prednisolone acetate had after 1–2 weeks a decreased thymic weight and morphologically a reduction in the thickness of the thymic cortex, but no sign of increased lymphocytolysis was found (Table 1).

For the thoracic duct the relative cell count (cells/μl) and total lymph cell flow (cells/hour) in control and thymectomized guinea pigs is shown in figs 1 and 2. Neonatally thymectomized guinea pigs showed when adult a significantly reduced lymphocyte count in lymph with about a 50 per cent reduction.

After the steroid injection both groups showed a fall in lymphocyte counts. Three hours after the injection the reduction was about 30 per cent for the controls and about 33 per cent for the thymectomized compared to the original levels and expressed as cells/hour. The reduction of the relative cell count was about 40 per cent in controls and about 33 per cent

Table 1 Effect of different corticosteroid preparations on thymus weight (mean weight)

	Steroid Preparation (Dose (mg/100 g B. w.) and time interval)	Body Weight (G)	Thymus Weight (MG)
A.	*Prednisolone succinate*		
	10–20 mg and 3 hours (3 animals)	250	335
	10–20 mg and 17 hours (3 animals)	391	347
B.	*Hydrocortisone*		
	8–24 mg and 1–4 days (8 animals)	288 ± 24	317 ± 30
C.	*Hydrocortisone acetate*		
	10–20 mg and 12 hours (4 animals)	283	421
	10–20 mg and 24 hours (4 animals)	271	336
	10–20 mg and 48 hours (4 animals)	310	311
D.	*Methylprednisolone acetate*		
	10 mg and 1 week (12 animals)	197 ± 10	204 ± 24
	20 mg and 2 weeks (8 animals)	203 ± 13	151 ± 25
E.	*Control animals* (15 animals)	306 ± 24	447 ± 27

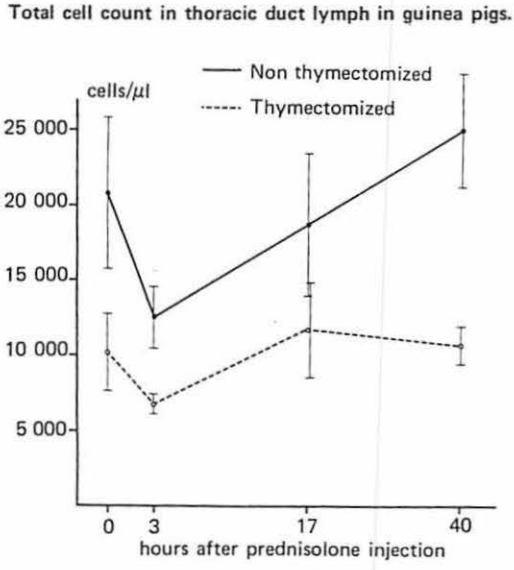

Fig. 1 The thoracic duct lymph cell count in normal and neonatally thymectomized guinea pigs at different times after a corticosteroid injection.

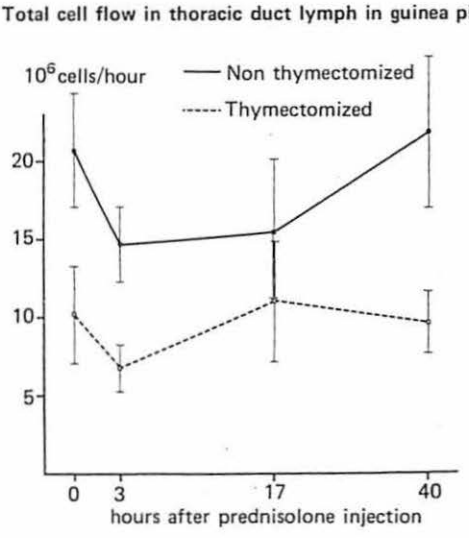

Fig. 2 The thoracic duct lymph cell flow in normal and neonatally thymectomized guinea pigs at different times after a corticosteroid injection.

in thymectomized animals. Restitution to pretreatment cell level in lymph was completed within 17–40 hours.

In venous blood the mononuclear cells were markedly depressed at both 3 and 17 hours and remained so also at 40 hours in the thymectomized group (fig. 3). The polymorphonuclear cells in venous blood rose at 3 hours and then fell below pretreatment levels (fig. 4).

Because of the age differences of the animals (10–18 weeks old at the time of the drainage)

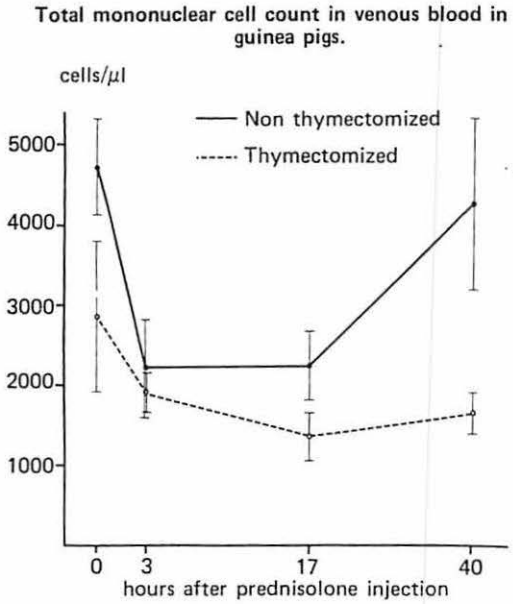

Fig. 3 The venous mononuclear cell count in normal and neonatally thymectomized guinea pigs at different times after a corticosteroid injection.

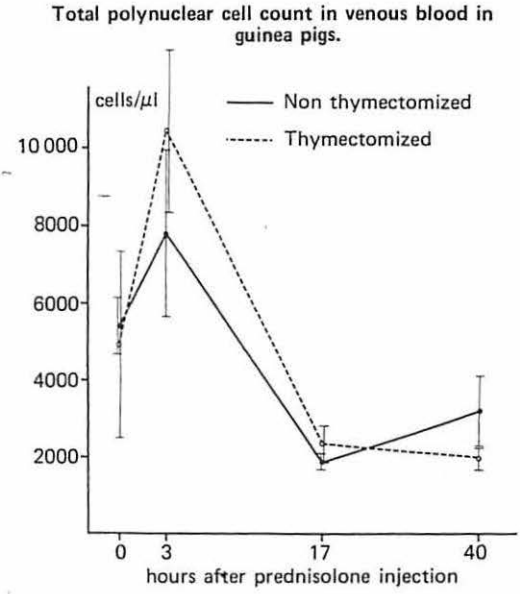

Fig. 4 The venous polymorphonuclear cell count in normal and neonatally thymectomized guinea pigs at different times after a corticosteroid injection.

Table 2 Body weights (G.) and organ weights (MG.) of normal and thymectomized guinea pigs at different times after a corticosteroid injection (mean ± standard error of the mean).

	Guinea Pigs	B.W.	Thymus	Spleen	Mesenterial Lymph Node	Other Lymph Nodes*	Adrenals
Nonthymectomized: untreated	5	576 ± 49	618 ± 104	786 ± 176	257 ± 62	154 ± 40	370
Nonthymectomized: 3 hours after steroid treatment	5	490 ± 57	509 ± 95	524 ± 114	335 ± 75	118 ± 35	158
Nonthymectomized: 17 hours after steroid treatment	5	506 ± 58	571 ± 132	547 ± 98	357 ± 83	122 ± 41	179
Nonthymectomized: 40 hours after steroid treatment	5	606 ± 111	561 ± 167	760 ± 174	390 ± 119	263 ± 91	389
Thymectomized: untreated	5	687 ± 75	–	842 ± 124	392 ± 140	232 ± 97	381
Thymectomized: 3 hours after steroid treatment	5	692 ± 126	–	659 ± 40	509 ± 172	229 ± 57	293
Thymectomized: 17 hours after steroid treatment	5	704 ± 142	–	667 ± 61	243 ± 41	258 ± 55	327
Thymectomized: 40 hours after steroid treatment	5	747 ± 135	–	727 ± 111	525 ± 108	169 ± 33	380

* includes axillary, para-aortic and thymic lymph nodes.

it is difficult to compare the body weights and weights of lymphoid organs. However, it does not seem to be any significant, it, differences between the groups after steroid treatment (Table 2), except for the reduction of thymic weight after treatment with depot

preparations of methyl-prednisolone acetate for 1–2 weeks.

Discussion

The effect of neonatal thymectomy in guinea pig has been discussed earlier by *Gyllensten* (8) who found an increase in the weight of lymph nodes after neonatal thymectomy. In the present work the differences in body weights and organ weights made interpretation difficult. The lymph node weights were higher in the thymectomized group, but not significantly so.

The circulating lymphocyte pool changed after neonatal thymectomy and showed a significant decrease in the cell level. Thus, the guinea pig is dependent on an intact thymus to keep the circulating cell level intact, analogous to what has earlier been reported for the rat and mouse. With regard to the blood lymphocytes our results agree with the data of *Ernström* and *Larsson* (9).

In the rat neonatal thymectomy causes a depletion of the thymus dependent areas in secondary lymphoid organs (6, 10). We could in this work find a slight depletion of the same areas also in the guinea pig, though not so marked as in the rat.

Histologically the animals showed no signs of increased lymphocytolysis in the thymus or in the spleen or lymph nodes after steroid injection. In thymectomized animals the slight depletion of thymus dependent areas remained during the involution and restitution phases. It is, however, possible to produce a decrease in guinea pig thymic weight with a reduction of the cortical thickness using a corticosteroid with depot effect or by repeated high doses of a short acting steroid for several days. It will take more than one week to produce these changes while in the mice an almost complete involution of the thymic cortex can be produced within two days with one large dose of prednisolone.

As judged by the lytic effect on lymphoid tissues the guinea pig seems to be very resistant to corticosteroids. However, the circulating lymphocyte pool is sensitive as measured by the disappearance of cells from the circulation. We found the same though less pronounced changes as for the rat. In both control and thymectomized guinea pigs the rapid disappearance of thoracic duct cells is followed by restitution to pretreatment levels within one or two days.

Earlier work by *Ernström* and *Larsson* (11) shows the same effect on mononuclear and polymorphonuclear blood cells, with a decrease of blood lymphocytes within 3 hours and an increase of granulocytes after steroid injection. The transient lymphocytopenia followed by a return to a normal cell count was also found by *Fauci* (12) who reported a greater decrease in blood T cells than in B cells. He also found a homing of ^{51}Cr-labelled lymphocytes in the bone marrow during the lymphocytopenic period. This agrees quite well with the changes found in mice during steroid involution (13, 14) and also with our hypothesis of trapping and redistribution of lymphocytes after a single steroid dose. Thus, the same mechanism, with a steroid sensitive circulating lymphocyte population, can be identified in both the steroid sensitive rat and the steroid resistant guinea pig in spite of the lack of lymphocytolysis in the resistant species. The changes are in principle the same whether the two species have a normal number of T and B cells or if they are depleted of T cells (neonatally thymectomized). The only difference between the two species appears quantitative with a more pronounced change in the steroid sensitive species. This may depend on a more pronounced redistribution of cells in the rat but an additional effect of lymphocytolysis can not be excluded.

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