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The Effect of Steroids on the Circulating Lymphocyte Population

I. Changes in the thoracic duct lymphocyte population of the rat after neonatal thymectomy and prednisolone treatment.

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Summary

The influence of prednisolone on the thoracic duct cells of rats was measured by the cell count in lymph at different times after a single injection and correlated with lymphocytolysis in lymphoid tissues.

In both control and neonatally thymectomized animals there was a marked fall in the cell count and flow at 3 hours and the percentage reduction was greater in thymectomized animals. Restitution was rapid in both groups and pretreatment levels were regained in 17 hours. The blood mononuclear cells followed the same pattern. In animals with an intact thymus adrenalectomy causes a significant increase in thoracic duct cell counts but in neonatally thymectomized animals thoracic duct lymph is unchanged after adrenalectomy. It can be concluded that in the rat lymphocyte level in the circulating pool is thymus dependent but restoration of the circulating cell count after steroid induced involution is independent of intact thymic function.

Our data support the hypothesis of lymphocyte trapping and redistribution as a major mechanism after a single cortisone dose.

Key-words: Lymphocyte – Thoracic duct – Lymphoid tissue – Blood – Corticosteroid – Thymectomy – Adrenalectomy – Rat

Introduction

The significance of the steroid sensitivity of lymphocytes and lymphoid tissue in species such as mice and rats is still unknown. Neither is the steroid sensitive population clearly defined relative to knowledge of functionally different lymphocyte populations, and little is known about a possible steroid effect on lymphocytes of so-called steroid resistant species such as man and the guinea pig. For reviews see *Claman* (1) and *Bach* (2).

The most pronounced morphological effect of steroids in sensitive species is seen in the thymic cortex which, after large doses, can be totally depleted of small lymphocytes. In peripheral lymphoid organs such as spleen, lymph nodes and Peyer's patches the most obvious effect occurs in the follicular germinal centers with pronounced lymphocytolysis and phagocytosis of pyknotic lymphocyte nuclei. Germinal centres disappear in one or two days. In other lymph node regions and in the spleen a lesser effect can be seen with lysis of a few cells in the periphery of follicles and subcapsularly in the lymph node cortex. A minimal effect is seen in the so-called thymus dependent areas described by *Parrott* et al. (3), which contain few steroid sensitive cells according to *Lundin* and *Järplid* (4). Bone marrow lymphocytes or at least the immunocompetent population is steroid resistant (5).

The relationship of these changes to immune system functions is not known. At least some of the functional changes in cell mediated immunity are also observed in steroid resistant species but large doses or prolonged treatment are required (1).

The modern classification of lymphocytes into T and B cells has been modified and clarified during recent years. Some authors have tried to define subpopulations of T and B cell lines. Thymus dependent lymphocytes are, for instance, divided into T1 and T2 cells by *Cantor* and *Asofsky* (6), and Th and Tp cells by *Konda* et al. (7). Definition of these T cell subgroups differ, but some common points emerge. The cell of one subgroup (T1, Th

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respectively) is steroid and radiation sensitive and morphologically represented by the thymus cortical cell, probably short-lived and poorly recirculating. The distribution and role in the peripheral pool is not clarified. No certain immunological function has been ascribed to it *in vivo*, but *Kappler* found that it may function as a suppressor cell (8)

The other subgroup (T2, Tp respectively) is steroid and radiation resistent. It is probably located in the thymic medulla and peripherally at least some cells are long-lived. These long-lived cells are the most numerous thymus dependent cells of the circulating pool and in the thymus dependent areas of lymph nodes, spleen and Peyer's patches. As judged by the capacity to induce a graft versus host reaction they are apparently immunocompetent as found by *Blomgren* and *Andersson* (9). They may function as a helper or suppressor cell in antibody production (8). The T memory cell (Tpm according to Konda) may be included in this group (7).

The relationship between these two T cell lines is unclear. They may represent two separate populations already in the thymus or the T1 cell line may differentiate peripherally to the T2 cell (10). The role of T lymphocyte subsets in different phases of the immune response have been discussed by, among others, *Cantor* and *Boyse* (11, 12) and *Peck* et al. (13). More recently, T cell subsets in mice has been characterized with the antigens of the Ly-series (11).

The situation is as unclear for the B cell. In the steroid sensitive species, the mouse, lymphocytolysis is observed also in B cell areas such as lymph follicles and especially in the germinal centres in all secondary lymphoid tissues (4). There is no evidence of more than one B cell development line. The heterogeneity of the B cell population is related to the differentiation of a stem cell to a virgin cell and further to an antibody producing cell or a memory cell (14). The mature antibody forming cells (e.g. plaque-forming cells) are certainly steroid resistant, but with administration prior to antigen exposure a primary antibody response is easily depressed with correct timing as described by *Berglund* (15). However, the antibody forming cells precursor in the bone marrow is not altered according to *Cohen* and *Claman* (5). The splenic precursor cells may be responsible for the steroid sensitivity of the humoral antibody response (5, 16). An influence on T-B cell cooperation cannot, however, be excluded.

Thus, in spite of the dramatic response in sensitive species, the steroid sensitive population is vaguely defined concerning morphology, kinetics and function. The possibility of a corresponding cell population in steroid resistant species, such as man and guinea pig, is not clarified.

The aim of the present work is to try to define the steroid sensitive lymphocyte population morphologically and functionally in relation to modern concepts of lymphocyte function. This paper describes the effect of prednisolone on the lymphocytes of the circulating pool, (i.e. the thoracic duct and blood), and in the lymphoid tissues of the rat (a steroid sensitive species), during acute steroid involution and restitution.

Material and methods

Rats of the Sprague-Dawley strain were thymectomized within 24 hours of birth. Thymectomy was performed under hypothermia and ether anaesthesia with partial sternotomy and vacuum extraction of the thymic lobes using a dissection microscope. Totally 143 animals aged 7–10 weeks, with body weights of about 200–300 g were used. Sex distribution was equal. Shamthymectomized (anaesthesia and sternotomy) and nonthymectomized rats served as controls.

The corticosteroid used was a water soluble preparation of prednisolone sodium succinate (Precortalon Aquosum, Organon). It was given intramuscularly in the thigh in a dose of 10 mg/100 g body weight. Some animals were injected with equal volumes of saline instead of prednisolone.

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* (17), was used. The

minimum requirements were drainage for 20 minutes and 0.1 cc of lymph, but usually drainage continued 60 minutes. The lymph was collected in heparinized glass tubes. Lymph volume was measured and a sample taken for mononuclear cell count. The total 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 counted. Lymph and blood smears were also made for microscopical examination. The animals were sacrified and the spleen, lymph nodes (thymic, para-aortic, axillary and mesenterial) and adrenals were dissected out, weighed and fixed in neutral formalin for histology.

Some of the neonatally thymectomized animals and corresponding controls were adrenalectomized as adults. A posterior approach was used under ether anaesthesia with aseptic conditions. Postoperatively the animals had saline to drink. Three or four days after adrenalectomy thoracic duct drainage was started as described above.

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

Results

The average relative cell count and total lymph cell flow in the thoracic duct of untreated controls (nonthymectomized and shamthymectomized animals) and of thymectomized untreated rats are shown in figs 1 and 2. Thymectomized rats showed an obvious significantly reduced lymphocyte count in the thoracic duct.

In both controls and thymectomized animals there was a marked fall in the lymphocyte counts 3 hours after the steroid injection. For the controls the lymphocyte reduction was about 50 per cent and for the thymectomized animals about 70 per cent of the original levels expressed as cells/hour. Seventeen hours after the steroid injection the original cell level was restored in both groups.

Mononuclear cells in venous blood followed a similar pattern in both groups with a

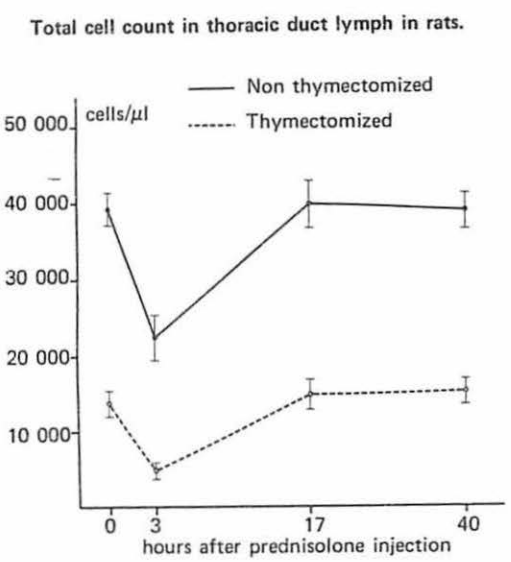

Fig. 1 The thoracic duct lymph cell count in normal and neonatally thymectomized rats at different times after a corticosteroid injection.

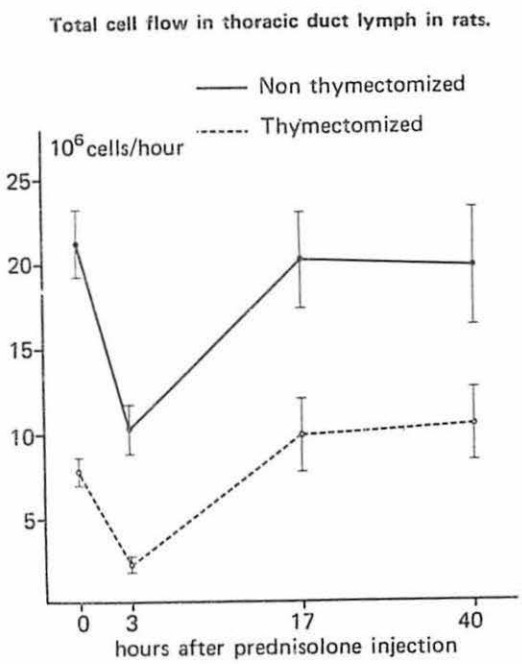

Fig. 2 The thoracic duct lymph cell flow in normal and neonatally thymectomized rats at different times after a corticosteroid injection.

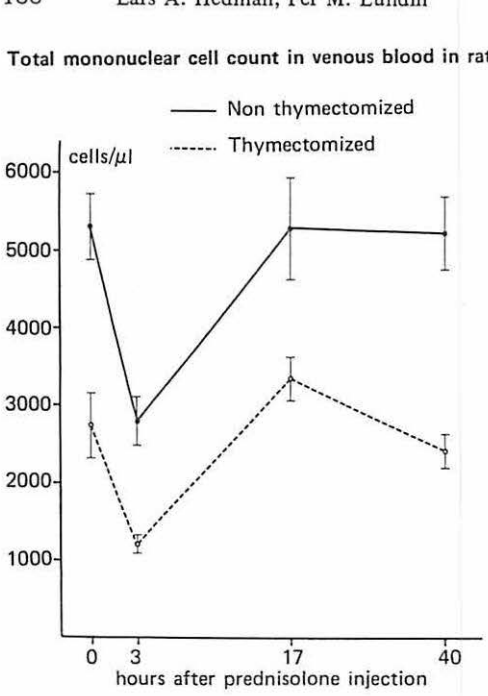

Fig. 3 The venous mononuclear cell count in normal and neonatally thymectomized rats at different times after a corticosteroid injection.

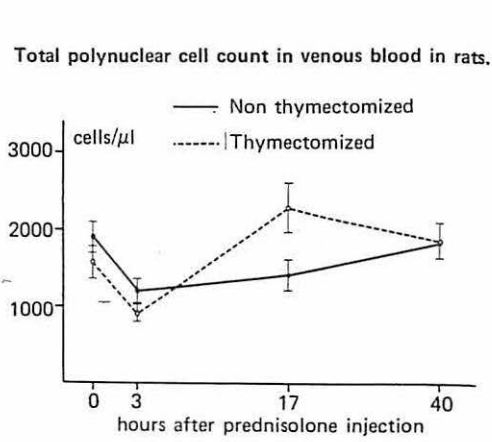

Fig. 4 The venous polymorphonuclear cell count in normal and neonatally thymectomized rats at different times after a corticosteroid injection.

marked fall 3 hours after steroid treatment and restoration within 17 hours. The polymorphonuclear cells in venous blood showed a slight similar tendency (figs 3 and 4).

The body, spleen and adrenal weights were the same in both untreated thymectomized and untreated control animals (Table 1). Mesenterial and other lymph nodes were smaller in the thymectomized animals and the

Table 1 Body weights (G.) and organ weights (MG.) of normal and thymectomized rats at different times after a corticosteroid injection (mean ± standard error of the mean).

	Rats	B.W.	Thymus	Spleen	Mesenterial Lymph Node	Other Lymph Nodes*	Adrenals
Nonthymectomized: untreated	28	262 ± 14	513 ± 40	792 ± 45	292 ± 13	151 ± 9	58
Nonthymectomized: 3 hours after steroid treatment	15	245 ± 8	421 ± 29	801 ± 110	290 ± 15	137 ± 10	64
Nonthymectomized: 17 hours after steroid treatment	15	263 ± 13	372 ± 31	971 ± 183	244 ± 20	139 ± 12	62
Nonthymectomized: 40 hours after steroid treatment	12	308 ± 18	323 ± 28	682 ± 40	310 ± 45	116 ± 7	49
Thymectomized: untreated	11	267 ± 17	–	734 ± 77	220 ± 12	125 ± 12	62
Thymectomized: 3 hours after steroid treatment	10	247 ± 14	–	767 ± 120	216 ± 18	124 ± 9	70
Thymectomized: 17 hours after steroid treatment	11	280 ± 16	–	883 ± 156	186 ± 15	121 ± 12	51
Thymectomized: 40 hours after steroid treatment	12	316 ± 16	–	663 ± 36	265 ± 66	93 ± 8	54

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

difference was not significant only for mesenterial lymph nodes. After steroid treatment body, spleen and adrenal weights were unchanged, but 40 hours after injection lymph node weights were lowered. In the control group the thymic weight was significantly decreased 40 hours after the corticosteroid injection (Table 1).

Histologically the spleen and lymph nodes of the thymectomized untreated animals showed a depletion of small lymphocytes in the so-called thymus dependent areas. Three hours after the prednisolone injection controls showed a marked lymphocytolysis in the thymic cortex, spleen and lymph nodes. In the lymph nodes cytolysis was seen chiefly in germinal centres and between follicles in the outer cortex. In the spleen lymphocytolysis was seen in the germinal centres. The thymus dependent areas of spleen and lymph nodes showed few pyknotic cells. Neonatally thymectomized animals showed a similar picture in the spleen and lymph nodes. Seventeen hours after the injection most of the pyknotic cells in the spleen and lymph nodes had disappeared in both groups. In the controls many pyknotic cells remained in the thymic cortex which was reduced in thickness.

Forty hours after injection the spleen and lymph nodes showed the same picture as at 17 hours, with few pyknotic cells. Follicles and germinal centres were smaller than in untreated controls.

In the nonthymectomized animals there was a significant rise in the lymph cell count and flow 3 days after adrenalectomy. Neonatally thymectomized animals showed no significant changes in the cell count in thoracic duct lymph 3 or 4 days after adrenalectomy (Figs 5 and 6).

Discussion

The rat is regarded a typical steroid sensitive animal with well documented morphological changes after steroid treatment as described by *Dougherty* (18). Using a corticosteroid preparation without depot effect the changes in the spleen and lymph nodes are quickly restored. The thymus shows marked changes, especially the cortex, where regeneration starts about the fifth or sixth day (19).

The reduction of circulating lymphocytes as effect of thymectomy performed at different ages are described earlier (20, 21) and our

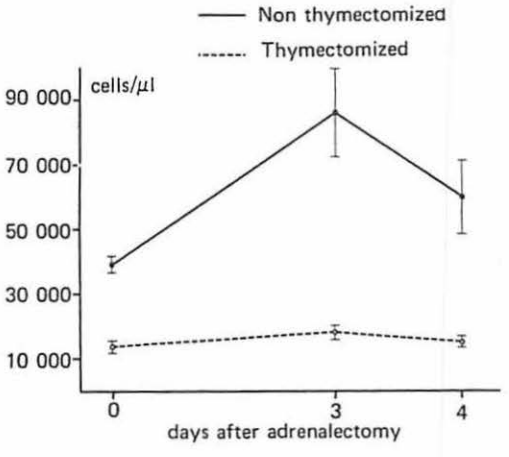

Fig. 5 The thoracic duct lymph cell count in normal and neonatally thymectomized rats at different times after adrenalectomy.

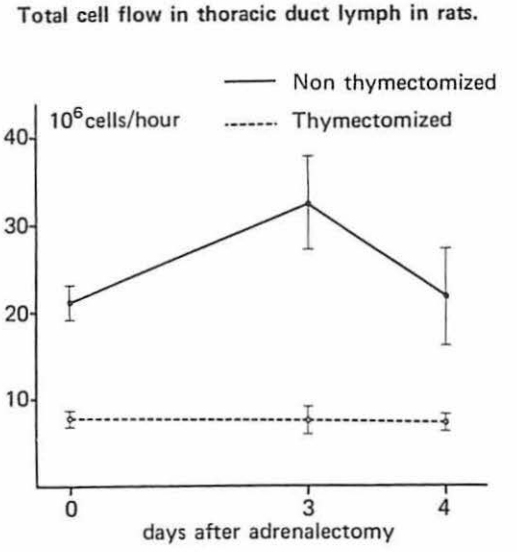

Fig. 6 The thoracic duct lymph cell flow in normal and neonatally thymectomized rats at different times after adrenalectomy.

results with neonatally thymectomized rats agree with these earlier observations. The effect of thymectomy concerning the completeness of T lymphocyte depletion is unclear. Animals neonatally thymectomized certainly lack most T cells but cannot be regarded as pure "B cell animals". Remaining T cell numbers depend on the time of thymectomy and the animals maturation. Neonatally thymectomized mice have up to 20 per cent theta positive cells in thoracic duct lymph and with further treatment by irradiation and bone marrow reconstitution that level can be depressed to about 10 per cent according to *Sprent* (22). However, T-B cell cooperation must be defective and alteration of the B cells cannot be excluded.

In the present work the changes in organ weights after neonatal thymectomy were the same as *Reinhardt* (23) found in 1945. In the spleen and lymph nodes we found the same effects as *Parrott* et al. (3) described in 1966, with lymphocyte depletion in the so-called thymus dependent areas. In thoracic duct lymph the steroid causes a rapid initial decrease of lymphocytes with rapid restitution to pretreatment levels within 17 hours. This rapid reappearance of circulating cells is seen both in thymectomized animals and animals with an intact thymus. Thus, the restitution of the circulating cell level after steroid treatment cannot be dependent on a direct cellular influence of the thymic cortex. In contrast the original level of lymphocytes in the circulating pool is thymus dependent.

Steroid injection seems to cause cytolysis chiefly in the thymus independent areas of spleen and lymph nodes both in neonatally thymectomized and control animals with a pronounced cytolysis seen after 3 hours. Thymus dependent areas of thymectomized animals are readily identified with only very few pyknotic cells after steroid treatment.

The microscopically observed effect of steroids on tissues, especially the thymus, is cytolysis, and the disappearance of circulating lymphoid cells has also been explained by cell death.

This predicted cytolysis can hardly be visualized in the circulating lymphocyte population, but it is known that peripheral lymphocytes of the rat are sensitive to steroids *in vitro* (21).

It is, however, difficult to connect a pronounced cytolysis with the rapid restitution of the circulating lymphocyte level. A possible alternative explanation could be a trapping of cells with redistribution to one or several organs. The bone marrow is one such possible organ and an increase of theta positive cells in bone marrow after steroid treatment in mice has been described by *Moorhead, Claman* (24) and *Cohen* (25). Infusion of ^{51}Cr-labelled lymphocytes in mice during steroid treatment also showed an accumulation in bone marrow (26).

The reappearance of cells in the circulation (both lymph and blood) can theoretically be due to a return of the original cell population after trapping, or to a rapid proliferation of new cells, or by a mobilization of cells from some depot. It can of course also be the result of a combination of these alternatives and to clarify that a careful analysis of the cell populations before, during and after steroid treatment is needed.

In the control animals with an intact thymus there was a significant rise in lymph cell number 3 days after adrenalectomy. This can be explained by the loss of endogenous cortisone secretion that normally depresses a steroid sensitive lymphocyte population in the circulating pool. Neonatal thymectomy combined with adult adrenalectomy produced no changes in lymph cell counts up to 4 days after adrenalectomy, which shows that this cell population is thymus dependent.

Our data support the hypothesis of lymphocyte trapping and redistribution as a major mechanism after a single cortisone dose.

Analysis of the lymphocyte populations during different phases of steroid action on the lymph of rats with a Coulter Counter and label indexing after isotope administration shows the same size distribution and same label index profile in all phases (*Hedman* and *Lundin*, to be published). These experiments would further support the hypothesis of lymphocyte trapping and redistribution as a major mechanism of corticosteroid action.

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