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Influence of Corticosteroids on Lymphocyte Recirculation

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Summary

The effect of corticosteroids on cell kinetics and cell size distribution in the circulating lymphocyte populations of the steroid sensitive rat and the steroid resistant guinea pig were studied.

A single high steroid dose (prednisolone) induced a rapid depression of the lymphocyte level both in normal and thymectomized animals of both species and a restitution within one day. The returning cell population showed the same size distribution and label index profile as before involution. The main effect of a single steroid dose seems to be a „trapping" of lymphocytes with redistribution from the circulation to some tissues. The difference between the two species seems to be quantitative with a more pronounced trapping mechanism in the sensitive rat but a certain degree of lymphocytolysis can not be excluded in this species. The steroid action seems to be on both T- and B-lymphocytes and the restitution of cell levels after acute involution is independent of an intact thymic function. The same course of events was observed in the normal rat after stress, but not in the thymectomized rat.

Corticosteroids of different types are often used in clinical medicine to influence immunological and inflammatory processes. In the field of oncology they have been used in the treatment of malignant diseases of the lymphoid system, mostly in combination with cytostatics. Much is known about the biochemical and morphological effects of steroids but, in spite of extensive clinical use for more than 30 years, the precise mechanisms involved in the corticosteroid effect remain unclear. The relationship between the corticosteroids' general metabolic effects (e.g. on protein synthesis and carbohydrate metabolism) and the depressant effect on lymphoid tissues and immune mechanisms has not been clarified.

It is generally agreed that the first step of the steroid action is controlled through cytoplasmic receptor proteins with affinity to the steroids. These receptors are not yet well characterized.

Depending on the cytolytic effect of corticosteroids on the lymphoid tissues, especially the thymic cortex, animals have been divided into sensitive species (mouse, rat, and rabbit) and resistant species (guinea-pig, monkey, and man). The functional effects on the lymphoid system and immune reactions are only partly correlated to this cytolytic effect. Thus, in mice and rats it is easy to induce depression of the cellular immunity, measured as a delayed type reaction or graft rejection. The humoral antibody production can also be depressed when corticosteroid administration is rightly timed in relation to the antigen injection. In the guinea-pig and man these corticosteroid effects are definitely weaker and higher doses are required to induce a response.

In spite of this weak effect on the immune reactions in the resistant species and the very low cytolytic effect on the thymic lymphocytes (perhaps absent in the guinea-pig), an observed on inflammatory reactions and in man, especially those connected with presumed autoimmune reactions e.g. rheumatoid arthritis and „allergic" vascular reactions. However, these effects can be at least partly explained by other corticosteroid mechanisms, such as a general catabolic effect, influence on macrophages and polymorphonuclear neutrophils, a depressant effect on fibroblasts and capillary endothelium, and an effect on vascular permeability (Rev. *Bach* 1975). Corticosteroids have also been said to stabilize lysosomal membranes, which might prevent the inflammation induced by release of lysosomal enzymes from monocytes and polymorphonuclear neutrophils.

Our present studies deal with the effect of corticosteroids on the kinetics of the peripheral lymphocytes in the circulating pool (i.e. thoracic duct lymph and blood). In these studies we are comparing the steroid-sensitive

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rat and the steroid-resistant guinea-pig. Both neonatally thymectomized animals and animals with intact thymus are used. We have also tried to analyze more physiological mechanisms influencing the endogenous corticosteroid secretion, such as physical stress and adrenalectomy.

The rat and guinea-pig were chosen for the experimental model because these animals are well suited for thoracic duct lymph drainage by the open neck technique. Neonatal thymectomy was performed 24 hours after birth. The corticosteroid preparation used was prednisolone sodium succinate, a water-soluble preparation without any depot effect (*Lundin & Schelin* 1966). The experiments were performed when the animals were 2–3 months old and the effect of the corticosteroid was studied immediatly before and 3, 17 and 40 hours after the steroid injection.

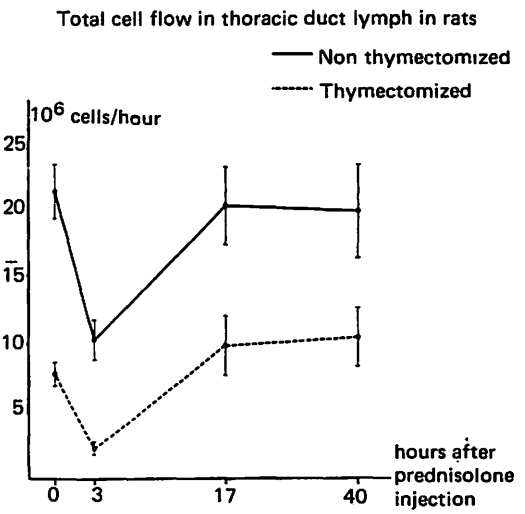

Fig. 1 The thoracic duct lymph cell flow in normal and neonatally thymectomized rats at different times after corticosteroid injection.

Steroid injection in the sensitive rat

Fig. 1 shows the lymphocyte flow per hour in rats at different times after the steroid injection. In the normal animals with an intact thymus a 50 per cent decrease is observed 3 hours after the injection and corresponding thymectomized animals have a 70 per cent depression in cell flow. Normal values are regained within 17 hours. The mononuclear cells in blood show the same changes as in the lymph. The polymorphonuclear cell level in the blood also shows an initial decrease, as may be seen in fig. 2. With the aid of a Coulter Counter, the size distribution of the thoracic duct lymphocytes has been registered during the different phases (i.e. the initial cell level depression and the subsequent cell level restitution). Fig. 3 shows the effect of neonatal thymectomy. A slight effect on the size distribution of lymph cells can be seen, with a more pronounced reduction of smaller cells. As shown in Fig. 4, no changes can be observed in the size distribution of thoracic duct lymphocytes in normal rats after steroid treatment and, further, the size distribution of returning cells is almost identical with that of untreated animals. The same changes occur in principle in thymectomized animals.

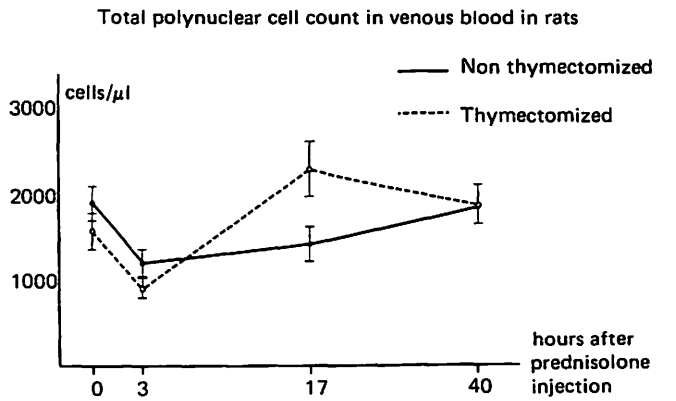

Fig. 2 The venous polymorphonuclear cell count in normal and neonatally thymectomized rats at different times after corticosteroid injection.

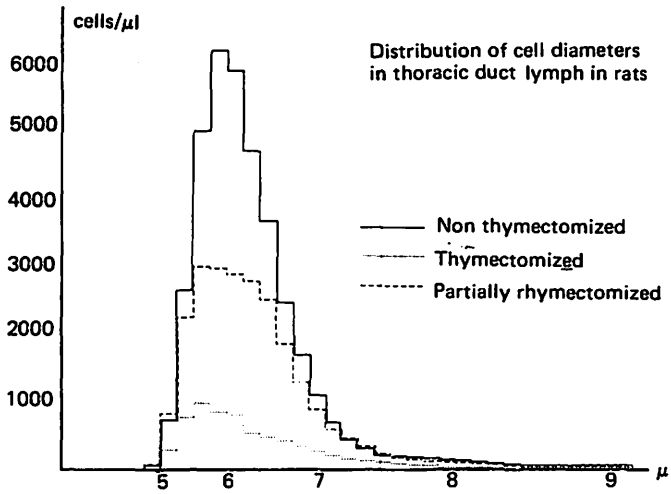

Fig. 3 The total cell size distribution in thoracic duct lymph in normal and neonatally thymectomized rats.

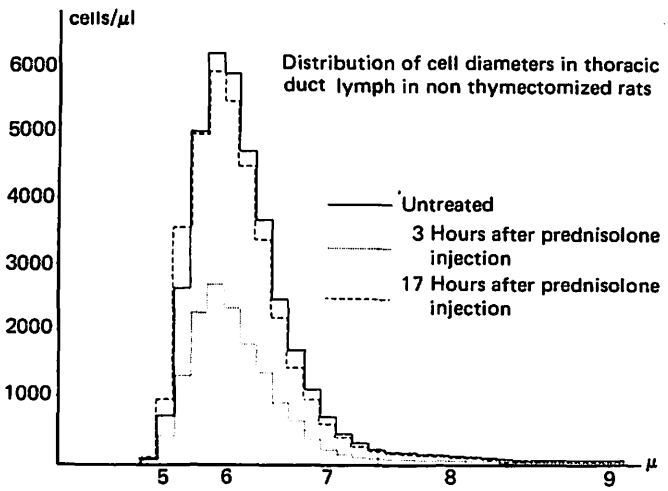

Fig. 4 The total cell size distribution in thoracic duct lymph in normal rats at different times after corticosteroid injection.

By labelling the lymphocytes in vivo with H^3-thymidine, we tried to study the age or maturation of the thoracic duct lymphocytes. The animals were heavily labelled with two daily injections for one week. By this procedure both short- and long-lived cells must be labelled. Steroid treatment the day after the last isotope injection did not give any significant change in the profiles during the involution and restitution phases (fig. 5). The same experiment performed 2 weeks after the last isotope injection, when only long-lived cells can be expected to have retained the label, show the same relative proportions of labelled small, medium, and large lymphocytes in both groups.

Steroid injection in the resistant guinea-pig

The same studies performed in the steroid-resistant guinea-pig gave the following results. After neonatal thymectomy, there was a 30 per cent reduction in the thoracic duct cell level. The steroid effect on thoracic duct lymphocyte flow was in principle the same as in the rat, but the cell counts are more variable and the restitution is perhaps somewhat slower. The blood mononuclear cells in the guinea-pig show the same involution and rege-

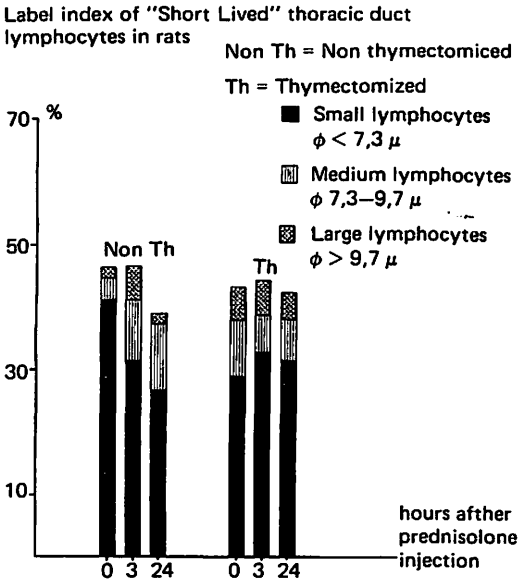

Fig. 5 Label index of "short and long- lived" thoracic duct cells in normal and neonatally thymectomized rats at different times after corticosteroid injection.

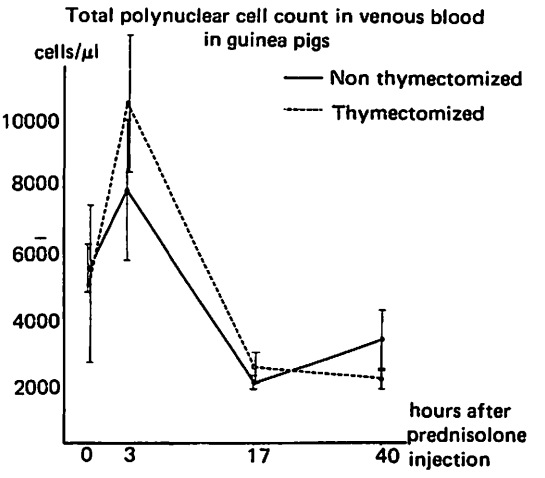

Fig. 6 The venous polymorphonuclear cell count in normal and neonatally thymectomized guinea-pigs at different times after corticosteroid injection.

neration changes. The polymorphonuclear blood cells in the guinea-pig show a short and significant initial increase in number (fig. 6).

The size distribution of the lymphocytes in thoracic duct in non-thymectomized guinea-pigs does not change under the influence of steroids, as shown in fig. 7, and the same was found for thymectomized animals.

Stress in the sensitive rat

The steroid doses used must be regarded as high. In attempt to find out whether the same steroid effect can be induced in a more physiological way, we exposed rats to physical stress by allowing them to swim in water at 30 degrees centigrade for half an hour. Fig. 8 shows that this amount of stress increased the level of corticosterone in the blood for a couple of hours in both intact and thymectomized animals, and the corresponding effect of this stress on the thoracic duct lymphocytes is shown in fig. 9. There is a significant cell level depression followed by a restitution in the intact animals and perhaps an overcompensation after two days. In the thymectomized animals, on the other hand, there is an insignificant, very slight depression 3 hours after the stress.

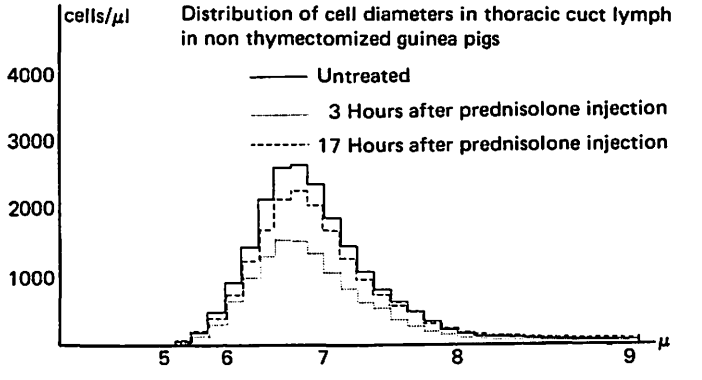

Fig. 7 The total cell size distribution in thoracic duct lymph in normal guinea-pigs at different times after corticosteroid injection.

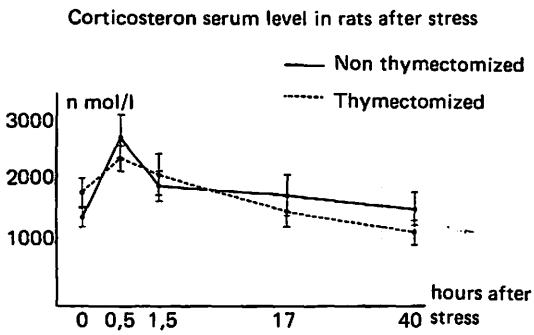

Fig. 8 The serum level of corticosterone in normal and neonatally thymectomized rats at different times after stress.

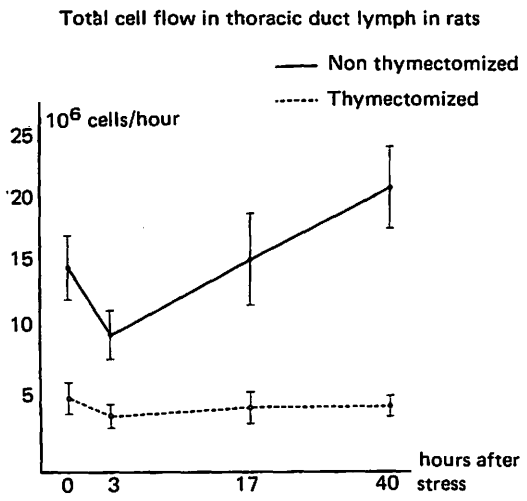

Fig. 9 The thoracic duct lymph cell flow in normal and neonatally thymectomized rats at different times after stress.

Adrenalectomy in the sensitive rat

Adrenalectomy may be regarded as the reverse of steroid treatment or stress. Fig. 10 shows that after adrenalectomy there is an significant increase in the number of thoracic duct lymphocytes. This increase, however, lasts only for a few days and might reflect a thymus-dependent steroidsensitive population that is normally depressed by the adrenals and stress mechanisms.

Summing upt the results of our studies (3–7), they indicate that an important mechanism in the induction of a peripheral lymphocytopenia after steroids in both the steroid-sensitive rat

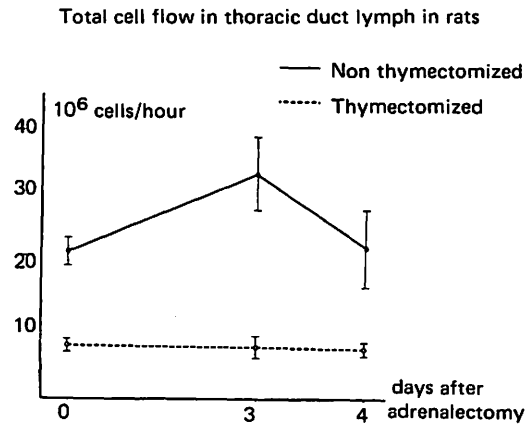

Fig. 10 The thoracic duct lymph cell flow in normal and neonatally thymectomized rats at different times after adrenalectomy.

and the steroid-resistant guinea-pig is redistribution of the cells from the circulation, followed by a return to pretreatment cell levels within one day. The returning cell population cannot be distinguished from the pretreatment one. It can be deduced from our results that both T- and B-lymphocytes are affected. Preliminary results of immunofluorescent studies in normal rats show no difference in the distribution of T- and B-lymphocytes during the involution and regeneration phases after steroid treatment.

The steroid effect is not a simple pharmacological one. It can also be elicited through an internal endogenous corticosteroid secretion after physical stress.

Our ideas on this matter are in accordance with some previously published results. *Fauci* (8) studied the blood lymphocytes in man, who is regarded as corticosteroid-resistant. He found a transient blood lymphocytopenia after 80 mg of prednisolone by mouth and observed a decrease of both T-cells (E-receptors) and B-cells (EAC-receptors) with the rosette-technique. *Cohen* (9) and *Moorhead & Claman* (10) have presented evidence for the redistribution mechanism. They showed that bone marrow may be at least one of the tissues in which these redistributed cells can "home" or be trapped. They observed an increase of theta-positive cells in the mouse bone marrow after steroid treatment, but such changes can of course be

due to other mechanisms than mere redistribution of cells. Infusion of Cr^{51}-labelled lymphocytes in the guinea-pig (11) has also revealed an accumulation of the isotope in the bone marrow after steroid treatment, but such experiments do not differentiate with certainty between living cells and phagocytized debris from disintegrating cells.

The relevance of these phenomena to the problems of neoplasia is at present difficult to assess. From a purely theoretical point of view, it can be speculated that this redistribution of lymphocytes may be a part of the complex mechanisms of cellular cytotoxicity. However, the relationship between induction and control of malignant diseases and cellular cytotoxicity in vivo is today very unclear.

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Discussion

Engeset: Is the effect of lymphocyte depletion caused by influence of steroid on the lymphocyte or on the blood capillaries. Do you know about any experiments where they have exposed lymphocytes to corticosteroid and reinjected them to see if they go the bone marrow?

Lundin: No, this will be the next step to treat the lymphocyte in vitro and see how they behave in vitro.

Ford: That would be an interesting experiment. In my opinion the marked increase in the number of recirculating lymphocytes in bone marrow following corticosteroid administration can be sufficiently accounted for by a decrease in the release of lymphocytes from the bone-marrow without any change in the influx from the blood. Recent work by G.H. Rannie and E.B. Bell (to be published) has emphasized the rapid transit and high volume of the lymphocyte traffic between the blood and bone-marrow. The mean transit time for T cells is only 2–3 hours, which means that the content of recirculating lymphocytes in marrow will double in about 2 hours if their release is stopped. Corticosteroids may influence the migration of lymphocytes from the blood into some tissues, but this has never been critically examined.