Influence of Ovariotomy on Recovery of Thymus Following X-ray Irradiation in ICR/JCL Mice

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Ovariotomy in 8 week-old, specific pathogen free ICR/JCL mice was effective in securing a marked thymus regeneration following a single whole body irradiation with 300 rads of X-ray. Recovery of the bone marrow cell counts, especially of the lymphoid cells, preceded the thymus regeneration and was more marked in spayed mice than in the sham-operated. On the basis of these results, it is suggested that ovarian hormones play a role in the thymic lymphopoiesis by affecting the dynamics of the lymphoid stem cells in the bone marrow and/or the lymphopoietic microenvironments in the thymic cortex.

INTRODUCTION

The thymus is highly sensitive to various stressful stimuli, usually resulting in an acute atrophy or involution (lymphocyte depletion) of various degrees in proportion to the intensity of the stimuli. Many of these stimuli, such as ionizing irradiation\(^1\), infections\(^2,3,4\) and malnutrition,\(^5\) act as lymphocytolytic agents either directly or indirectly via the enhanced release of adrenal glucocorticoids. Thymic involution has also been described as a normal accompaniment of pregnancy and lactation\(^6,7\).

Apart from the decisive role of glucocorticoids in the thymus atrophy, little is known concerning the effect of sex steroids upon the thymic lymphopoiesis. Several studies have demonstrated that male and female sex steroids give rise to changes in thymus weight and thymic lymphocyte proliferation\(^6,8,9,10\). Their mode of action is uncertain but they appear equally effective even in the absence of the adrenals, suggesting no participation of adrenal cortex in mechanism of the steroid action\(^5\). A marked sex difference in thymus weight has been found in most strains of mice; female thymus is usually greater than that of male thymus in both relative and absolute weight after adolescence\(^11\).

The thymus becomes lymphoid late in foetal life, and the organ rapidly increases in size after birth, reaching a maximum weight between the fourth and sixth weeks of age. After this period a characteristic loss of weight occurs (age involution) which proceeds rapidly for the first few months, then continues at a very much slower rate in later life\(^5\).

However, it is still obscure whether the age involution of the thymus proceeds under the influence of fluctuating hormonal factors such as glucocorticoids and sex steroids. Some workers claim a superior role of the thymus age which is intimately associated with the productivity of putative lymphocytopoietic thymic factor(s)\(^5\).

The present experiments were designed to study the influence of sex steroids at...
physiological level (in vivo) on the thymic lymphopoiesis in mice, originally being motivated by the observation that ovariotomy in mammary-tumor bearing rats was followed by a marked thymus enlargement.

MATERIALS AND METHODS

Experimental animals: A hundred of female ICR/JCL mice (specific pathogen free) were purchased from CLEA, Ltd., Osaka, Japan. They were housed in plastic cages, 10 in each and fed with commercial diet (Oriental MF) and water ad libitum. Veterinary aureomycin was given to the animals by drinking water for 1 week after operation.

Treatments: Half of the animals were ovariotomized at 8 weeks of age and the remainings were sham-operated under the Nembutal plus ether anesthesia. One day after the operation 300 rads of x-ray was given to whole body; physical factors being 200 KVp, 44 rads/min., HLV, 1.18 mm Cu, 0.5 mm Cu + 1 mm Al filters, target skin distance 60 cm. Two to four mice from each group were sacrificed to examine body weight, the number of white blood cell and red blood cell collected from the tail vein at intervals of 1-35 days after irradiation. The right femoral bone was removed and cut off at both epiphyseal ends. Then, by using 1 ml tuberculin syringe with 1/1 needle the whole marrow content was thoroughly washed out with Eagle MEM plus 10% rat serum in to the small petri dish and squeezed several times to make homogeneous cell suspension for cell counting. Bone marrow cell preparation for cytological study was made by centrifugating the cell suspension at speed of 900 rpm for ten minutes. Thymus, spleen, mesenteric lymphnode, uterus, liver and kidney were weighed by torsion balance (sensitivity 1 mg) immediately after the resection. Bone marrow cell preparations were stained with MayGrunwald Giemsa. The tissue sections were fixed in Bouin's solution, embedded in paraffin and stained with hematoxylin and eosin (H. and E.).

RESULTS AND DISCUSSION

Changes in body weight and organ weights: There was no substantial difference in body weight between the ovariotomized and sham-operated mice except for the animals sacrificed 4 weeks after irradiation.

In sham-operated mice uterus weight showed a sharp rise at 2nd week following an initial drop after irradiation. On the other hand, a steady decline of uterus weight was observed in ovariectomized mice.

Thymus was decreased in weight immediately after irradiation, from 0.35% to 0.1% of the body weight in both experimental and control mice (Fig. 1.). The weight decrease continued until 7 days after irradiation. Thereafter, a relatively rapid recovery was seen only in ovariotomized animals and the thymus weight reached to the pre-irradiation level by 2 weeks. Statistical analysis (t test) showed a significant difference between the two groups at 4 and 5 weeks.

There was no significant difference in the spleen weight between the spayed and the sham-operated mice. In mesenteric lymphnode, liver and kidney there were no differences between the two groups.

Changes in peripheral blood cell counts: Peripheral blood examination revealed no marked difference in both red blood cell and white blood cell counts between spayed and sham-operated control mice. However, a more pronounced lymphocytosis was ob-
served in ovariotomized mice 3 to 5 weeks after irradiation.

**Mitotic indices in thymus cortex:** Lymphocytopoietic activity of the thymus was studied by counting mitotic cells in the cortex (H. and E.). Changes in the number of mitotic cells per 1000 thymic lymphocytes are shown in Fig. 2. Mitotic indices were higher in ovariotomized mice for 7-35 days after irradiation.

**Changes in nucleated bone marrow cell counts:** Bone marrow cells responded very rapidly to a single whole body irradiation, i.e., cell counts on the 2nd postirradiation day dropped to $3 \times 10^6$/femur and resumed to proliferate on the 5th postirradiation day in spayed mice, while the recovery of bone marrow cell counts was far more retarded...
in the sham-operated animals (Fig. 3).

DISCUSSION

Weight of the thymus gland is determined mainly by interaction between production and emigration of the lymphocytes\(^5,12\). Furthermore, inflow of the lymphoid stem cells...
into the thymus affects the thymus regeneration following a whole body irradiation or thymus grafting\textsuperscript{5,13,14,15}).

In order to understand the mechanism of weight changes in the thymus, the present authors have hypothetically presented that there are three sites of actions of the ovarian hormones as follows: 1. lymphoid stem cells in the bone marrow, 2. lymphocyte proliferation in the thymus cortex, and 3. lymphocyte release or destruction.

According to the results obtained from the present experiments, recovery of the bone marrow cell level precedes the thymus regeneration, being more marked in spayed mice than in the sham-operated (Fig. 4). Interestingly, number of the lymphoid bone marrow cells has remarkably increased in the spayed, corresponding to the accelerated increase in thymus weight (Photo. 1). However, direct evidence of the immigration of the lymphoid cells to the thymus has not yet been obtained.
Estrogen suppresses erythropoietin production, resulting in inhibition of erythroid cell proliferation\(^\text{16}\). On the basis of the present results we presume that estrogen might act directly as one of the modifiers of lymphoid population in the bone marrow or indirectly by affecting the production or activation of "lymphocytopoietic substance". The latter case can also be associated with thymocyte production in the thymus cortex. Daily injections of 10 mg of estradiol benzoate into the male rat is equally effective in causing a profound thymic involution as compared to the testosterone treatment\(^\text{10}\). Nevertheless, both sex hormones can not induce an acute involution with a single injection\(^\text{10}\), whereas hydrocortisone has an acute lymphocytolytic effect on thymus\(^\text{8,17}\).

According to the study on the thymus regeneration in hydrocortisone-treated mice, there is no substantial difference between the spayed and normal female animals in respect of the recovery pattern of the thymus\(^\text{18}\). The previous workers did not refer to the bone marrow injury by hydrocortisone, which might be different from that caused by X-ray irradiation. An acute thymic involution after a single whole body irradiation with X-ray was not prevented by bilateral adrenalectomy (Takizawa et al., unpublished data).

It is generally accepted that gonadectomy does not prevent the age involution of thymus in male and female mice\(^\text{19}\). Neonatal thymus grafted into the old animal has more potential for growth than the in situ thymus\(^\text{9}\). These facts favored the assumption of intrinsic factor (s) which might regulate the thymic lymphopoiesis and eventually the thymus weight\(^\text{5}\).

Nevertheless, we cannot ignore the subclinical infections in conventional mice, which commonly affect the thymus environments very acutely. In many cases of unreasonably tiny thymus found at autopsy, microscopic and more preferably microbiological examinations disclosed the presence of focal infections. Therefore, the experiments on thymus lymphopoiesis using conventional animals may sometimes mislead the results to the erroneous interpretation. In this sense the use of germ free mice should be indispensable for the accurate analysis of the thymus weight changes.

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REFERENCES