Effects of Pregnancy and Lactation on Lymphocytes in the Bone Marrow of the Mouse: A Quantitative Electron Microscopic Study*

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Summary. Effects of pregnancy and lactation on lymphocytes in the bone marrow of the mouse were quantitatively examined by electron microscopy.

Pregnancy induces a decrease in the frequency of marrow lymphocytes, and delivery causes further depletion of marrow lymphocytes. Then the frequency of lymphocytes gradually increases to normal 20 days after delivery. The frequency is restored to the normal level earlier in non-lactating than in lactating mothers.

Marrow small lymphocytes, which constitute the majority of marrow lymphocytes, are distinguishable by electron microscopy into dark and light types. During pregnancy, the two types of small lymphocytes are equally decreased in proportion. Just after delivery dark small lymphocytes decrease markedly and thereafter increase gradually during lactation. Light small lymphocytes exhibit a gradual increase in proportion during lactation and reach the normal level at 20 days postpartum.

The results are discussed as compared with the effect of pregnancy and lactation on the lymphatic tissues, central and peripheral.

The bone marrow is generally included in the central lymphatic tissue with respect to its lymphocyte production. Recently we have reported that marrow lymphocytes show significant age- and sex-related changes and that the changes in marrow lymphocytes are similar in pattern to those in the thymus, the other central lymphatic tissue (SasakI and Ito, 1978, 1980). In relation to sex, it has been observed by earlier investigators (Persike, 1940; Gregoire, 1947; Ito and Hoshino, 1962) that pregnancy and lactation exert significant effects on the thymus. However, no information is available as to whether or how marrow lymphocytes are affected by pregnancy and lactation. In this study, therefore, it was undertaken to examine, by electron microscopy, lymphocytes in the mouse marrow during pregnancy and lactation. In the marrow, as pointed out previously (SasakI, 1976; SasakI and Ito, 1978), lymphocytes can be definitely identified and differentiated by electron microscopy rather than by light microscopy.

MATERIAL AND METHODS

Sixty-eight female mice of dd-strain were used in this study. The mice were maintained on commercial standard pellets and water provided ad libitum under constant environmental conditions. Female mice, 60 to 70 days old, were caged with males overnight, and they were examined next morning for the presence of a vaginal plug. The day of finding the vaginal plug was designated 0 day of gestation. The females were killed at 5, 10 and 15 days of pregnancy, and 0 (within 12 hrs), 5, 10 and 20 days after parturition. The mothers after parturition were divided into two groups; one group was composed of lactating mice which were allowed to suckle their young, and the other consisted of non-lactating mice from which the litters were removed just after delivery. Virgin females at 60 days of age served as normal controls. For each group at each period more than five mice were used.

At autopsy, mice were killed with excess chloroform, and the femurs were quickly removed, and cut open longitudinally by razor blades. The split femurs with marrow were placed in 5% formalin in 0.05 M phosphate buffer (pH 7.5) containing 1.5% sucrose for 48 hrs at 4°C. Then pieces of the marrow were gently removed from the bone tissue, washed in phosphate buffer, and postfixed in 2% OsO₄ in the same buffer solution for 2 hrs at 4°C. Some pieces of the marrow were fixed in 5% formalin–1% glutaraldehyde in 0.1 M cacodylate buffer at pH 7.5 for 2 hrs, and postfixed in 2% OsO₄ for 2 hrs at room temperature. The tissues were stained in block overnight with 0.5% uranyl acetate, dehydrated in graded ethanols, and embedded in Epon 812. Ultrathin sections were cut, stained with lead citrate, and examined with a Hitachi HS-9 electron microscope.

The method for quantitative analysis was the same as described in our previous papers (Sasaki, 1976; Sasaki and Ito, 1978, 1980). The differential counts of nucleated hemopoietic cells were made on micrographs enlarged to a final magnification of 3,500 times. For each mouse, more than 1,000 nucleated blood cells in the marrow were counted, and the frequency (%) of each hemopoietic cell series and the proportion of lymphocytes classified were obtained. All the values obtained were statistically analyzed by Student’s t test.

RESULTS

Frequency of hemopoietic cell series

The frequencies of the hemopoietic cell series in the bone marrow of virgin, pregnant, and lactating females are shown in Figure 1. In normal females, lymphocytes are 14.9% of the total nucleated hemopoietic cells; erythroid cells, 34.4%; granuloid cells, 48.7%; megakaryocytes, 0.4%; and plasma cells, 0.2%.

In pregnancy, lymphocytes constitute 6.5% of all the nucleated hemopoietic cells at 5 days of gestation, 7.9% at 10 days, 8.2% at 15 days, and 8.2% at parturition. Thus marrow lymphocytes are significantly lower in frequency in pregnant mice than in normal mice (P<0.002). They undergo a further decrease after parturition, being
Marrow Lymphocytes during Pregnancy and Lactation

4.4% at 5 days. Then, however, lymphocytes show a progressive increase in frequency until the normal level is restored at 20 days postpartum.

Erythroid cells gradually increase in frequency during pregnancy and reach a peak at 15 days of gestation. They return to almost the normal level after parturition. Granuloid cells decrease in frequency during pregnancy, but they gradually increase after parturition until almost the normal value is reached at 20 days. Megakaryocytes remain 0.6 to 1.0% of the marrow nucleated free cells throughout pregnancy and lactation. Plasma cells remain almost unchanged in frequency, 0.1 to 0.3%, during pregnancy and lactation.

The frequencies of the hemopoietic cell series in non-lactating mothers are presented in Figure 2. As shown in this figure, marrow lymphocytes constitute 2.5% of all the nucleated hemopoietic cells 5 days after parturition, but they are rapidly restored up to almost the normal level as early as 10 days after parturition. Thus, as shown in Figure 3, the frequency of marrow lymphocytes on the 10th day postpartum is significantly higher in non-lactating females than in lactating females (P<0.001).

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Fig. 3. Frequencies of marrow lymphocytes. C 60-day-old control. Each point represents mean, and bars indicate standard deviations.

Fig. 4. Bone marrow of a normal 60-day-old female. Two types of marrow small lymphocytes, dark ($D$) and light ($L$), were seen among other hemopoietic cells. Formalin and glutaraldehyde fixation. $\times 3,500$
Proportion of marrow small lymphocytes

As reported in previous papers (Sasaki and Ito, 1978, 1980), the majority of marrow lymphocytes are small lymphocytes, although larger lymphocytes are contained in very small numbers. In the normal marrow, larger lymphocytes constitute only 0.2% of all the nucleated hemopoietic cells and 1.6% of marrow lymphocytes. During pregnancy and lactation, the frequency of larger lymphocytes remains very low and almost unchanged. Thus changes in frequency of marrow lymphocytes during pregnancy and lactation are due to those in small lymphocytes.

As shown in previous papers (Sasaki and Ito, 1978, 1980), marrow small lymphocytes can be classified by their ultrastructural features into two types, dark and light small lymphocytes (Fig. 4). The proportions of the two types of small lymphocytes during pregnancy and lactation are presented in Figure 5. In virgin females aged 60 days, dark small lymphocytes constitute 51.4% of marrow small lymphocytes, and light small lymphocytes are the remaining 48.6%. During pregnancy, as mentioned above, lymphocytes decrease in frequency, but two types remain little changed in proportion. After parturition, however, dark small lymphocytes decrease, and they are 12.5% of marrow small lymphocytes at 5 days. Thereafter dark small lymphocytes...
gradually increase again to constitute 34.0% at 20 days postpartum. Light small lymphocytes, on the contrary, increase in proportion after parturition, and they occupy 87.5% 5 days and 66.0% of small lymphocytes 20 days after delivery. The proportions of the two types of small lymphocytes in non-lactating mothers are presented in Figure 6. The percentage of dark small lymphocytes is significantly higher in the non-lactating group than in the lactating group 10 days after parturition (P<0.001). On the 20th day postpartum the proportions of the two types of small lymphocytes are not significantly different between the lactating and non-lactating groups.

DISCUSSION

Effects of pregnancy and lactation on the lymphatic tissue, central and peripheral, have been studied for many years. Regarding the effects on peripheral lymphatic tissues, it has recently been reported that lymph nodes, particularly the ones that drain the uterus, increase in weight during pregnancy, especially the inter-strain ones (Allen and McLean, 1971; Maroni and de Sousa, 1973; Hetherington and Humber, 1977; Ansell et al., 1978; Forster et al., 1979), and that the regional lymph nodes, which have enlarged during pregnancy, return to normal after delivery (McLean et al., 1974). Thus the pregnancy-induced changes in peripheral lymphatic tissues are generally explained primarily in view of the alloantigenic status of the mother to embryos, particularly allogeneic ones (Maroni and de Sousa, 1973; McLean et al., 1974; Ansell et al., 1978). Concerning the effects of pregnancy and lactation on the central lymphatic tissue, it has been reported that the thymus undergoes involution in late pregnancy (Persike, 1940; Gregoire, 1947; Pepper, 1961; Ito and Hoshino, 1962; Millar et al., 1973; Clarke, 1979), and that the thymus becomes extremely involuted at parturition and remains atrophic during lactation (Ito and Hoshino, 1962; McLean et al., 1974). As is generally accepted, the central lymphatic organ, such as the thymus, does not directly respond to antigenic stimulation, but reacts to various endocrine factors (review: Weiss, 1972). In particular, the thymus is so sensitive to adrenocortical hormone that it is acutely involuted following increased adrenocortical activity such as is seen in stress situations (review: Dougherty, 1952). From these considerations, changes in the thymus during pregnancy have been thought to be primarily associated with endocrine factors rather than the immunological state of the mother (Pepper, 1961; Ito and Hoshino, 1962; Maroni and de Sousa, 1973). The pregnancy-induced involution of the thymus further goes on just after delivery and persists during lactation (Ito and Hoshino, 1962). In this relation, it has been previously detected that during lactation the adrenal cortex exhibits marked changes apparently correlated with its increased secretory activity which appears to reflect on the thymus (Ito et al., 1964).

As is well known, the bone marrow, like the thymus, is included in the central lymphatic tissue. In previous papers (Sasaki, 1976; Sasaki and Ito, 1980), we have reported that lymphocytes in the mouse marrow are sensitive to adrenal corticoids and sex hormones, particularly androgens, and that the response of marrow lymphocytes is similar in nature to that of thymic lymphocytes.

As shown in the results, the frequency of marrow lymphocytes is reduced as
early as 5 days of pregnancy and remains low during pregnancy. Then delivery causes further depletion of marrow lymphocytes. Marrow lymphocytes are least in frequency 5 days after parturition, and then they gradually increase to reach the normal level 20 days after parturition. On the other hand, when the mother has been separated from the young after parturition, the frequency of marrow lymphocytes is restored to the normal level earlier than that in lactating females. Thus lactation exerts a suppressive effect on marrow lymphocytes.

As reported previously, marrow small lymphocytes, which are more than 97% of the total marrow lymphocytes, can be classified by electron microscopy into two types, dark and light (Abe et al., 1973; Sasaki, 1976; Sasaki and Ito, 1978, 1980). The two types of small lymphocytes are different not only in ultrastructural features but also in certain functional properties, such as sensitivity to adrenocortical hormone and androgen (Sasaki, 1976; Sasaki and Ito, 1980). From comparison of small lymphocytes between the marrow and thymus it is assumed that dark small lymphocytes in the marrow are similar to cortical small lymphocytes in the thymus, and light ones, to medullary small lymphocytes, not only in morphologic features but also in nature (Abe et al., 1973). During pregnancy the two types of small lymphocytes are equally decreased in proportion. After delivery, however, dark small lymphocytes decrease markedly, and light ones exhibit a relative increase in proportion. In nonlactating females, marrow lymphocytes, particularly dark small lymphocytes, rapidly increase after parturition and quickly reach normal proportions. Thus dark small lymphocytes are particularly subject to the effect of lactation.

The initiation and maintenance of milk secretion following parturition are generally considered to depend on hormonal activities (review: Linzell, 1959; Topper, 1970). In particular, adrenal cortical hormones are thought to play a very important role in the development of the mammary gland (review: Cowie, 1972), and the plasma cortisol concentration is significantly higher in preparturient and lactating animals than in pregnant animals (Patterson and Linzell, 1971, 1974). As reported in a previous paper (Sasaki, 1976), dark small lymphocytes in the marrow are sensitive to, and light ones are resistant to hydrocortisone. Thus it is probable that decrease of dark small lymphocytes in the marrow after parturition and during lactation is caused by a rise in adrenocortical activity.

In conclusion, changes induced by pregnancy, delivery and lactation appear to be basically similar in nature for the central lymphatic organ, thymus and marrow, and they are thought to be associated with the hormonal rather than the immunological state. On the contrary, pregnancy-induced changes in the peripheral lymphatic tissue are no longer seen after delivery, and they are considered as representing an immunological response in pregnancy (Maroni and de Sousa, 1973; McLean et al., 1974; Ansell et al., 1978).

妊娠および授乳のマウス骨髄リンパ球におよぼす影響：電子顕微鏡による定量的研究

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妊娠および授乳期のマウス骨髄リンパ球を、電子顕微鏡を用いて定量的に観察した。
骨髄リンパ球の発現頻度は妊娠によって減少し, 分娩直後さらに減少を示す。その後,頻度は徐々に増加し, 分娩後20日で正常値に復する。分娩後では, 授乳群と非授乳群にわけると, 発現頻度は非授乳群で授乳群に比べて急速に回復する。

骨髄リンパ球は大多数が小リンパ球で占められるが, 小リンパ球は暗・明2型に区別される。妊娠によって, 暗・明両型ともに同様に発現頻度が減少する。暗調小リンパ球は分娩直後とくに著明に減少するが, 以後授乳期において徐々に増加する。一方, 明調小リンパ球の比率は, 分娩後授乳期で増加するが, 分娩後20日で正常に復する。

成績を, 他の中枢および末梢リンパ組織に対する妊娠および授乳の影響と比較して考察した。

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