Suppression of Lactation by Pregnancy-Dependent Mammary Tumors in GR/A Mice (42951)

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Abstract. **Pregnancy-dependent mammary tumors (PDMT) in GR/A mice appear during pregnancy, disappear soon after parturition, and appear again during subsequent pregnancies. The retardation of pup growth, an indication of the level of milk production, was also observed with the advance of lactation numbers in this strain. This study was performed to elucidate the relationship between PDMT and lactational performance. At the end of the second pregnancy, mice were divided into two groups according to the presence of PDMT [PDMT(−) and PDMT(+) groups]. Although all PDMT disappeared within a day after parturition, the weight and growth of pups on Day 12 of lactation were significantly less in the PDMT(+) group than in the PDMT(−) group. Associated with this, the DNA and RNA contents of the mammary glands were apparently lower in the former than in the latter, although the differences were not statistically significant. There was little difference in mammary RNA/DNA ratio between groups. No difference was also observed between groups in endocrine organ weights, mother body weights, morphology of the mammary glands, adrenals and ovaries and plasma prolactin and progesterone levels. These results suggest that PDMT suppression of lactation is principally due to the retardation of mammary gland growth. Furthermore, no significant correlations were obtained between the size of PDMT and the parameters for mammary gland function. The data suggest that the development of PDMT per se is important for the retarded mammary gland growth.** [P.S.E.B.M. 1989, Vol 192]

The GR/A mouse is characterized by the development of pregnancy-dependent mammary tumors (PDMT) as well as mammary hyperplastic alveolar nodules as a preneoplastic state of mammary cancers. PDMT appear after the middle of pregnancy, reach maximal size at the end of pregnancy, and regress and disappear soon after parturition regardless of lactation (1, 2). PDMT appear again at the subsequent pregnancies and the incidence, size, and number of lesions often increase with each additional pregnancy (2). It has also been observed that in the GR/A mouse lactational performance decrease with each subsequent pregnancy (3). These results led us to examine the influence of the development and/or progression of PDMT on mammary gland function in the GR/A mouse.

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Materials and Methods

Animals. GR/A mice were maintained in our laboratory by strict brother × sister mating. At 45–50 days of age, three to four females from each litter were mated with a male. Pregnant mice were housed individually and placed again with males only near parturition to induce concurrent pregnancy. Litter size was adjusted to five to six on the day of parturition (Day 0 of lactation) and nursed until Day 20 of lactation when weaned. Only mice that delivered concurrently at the second parity were used in the experiment. Incidences of PDMT were 9.5% and 57.1% at the first and the second parities, respectively. The high incidence of PDMT during the second pregnancy is why we used mice in the second lactation in this study. Mice were divided into two groups according to the presence of PDMT [PDMT(−) and PDMT(+) groups]. Litter size was adjusted again to five to six, weighed, and nursed until Day 12 of lactation. After each litter was weighed on the morning (8:00 AM–9:00 AM) of Day 12, lactating mice were killed by decapitation under the light ether anesthesia.

Throughout the experiment, mice were kept in plastic cages (16 × 28 × 13 cm) with wood shavings, maintained in an animal room which was air-conditioned (22–24°C and 55–70% relative humidity) and artificially illuminated (14 hours of light from 5:00 AM to 7:00 PM), and provided with a commercial diet (Lab MR Breeder; Nihon Nosan Kogyo KK, Yokohama, Japan) and tap water *ad libitum*.

Development and Progression of PDMT. Each mouse was palpated on Day 19 of the second pregnancy and the number and size of PDMT, which was expressed in terms of the mathematical mean of the major two diameters, were recorded. When more than two PDMT developed in a mouse, sum of the sizes of all PDMT was used as the representative value of the individual.

Reproduction. Delivery interval, litter size, stillbirth rate, rate of still-born pups, and rearing rate on Day 12 were employed as the indices of reproduction (4).

Lactational Performance. Average pup weight obtained by the division of litter weight by the number of pups and percentage of change in pup weight or pup growth rate on Day 12 were employed as the indices of lactational performance.

Mammary Nucleic Acid Contents. At autopsy, the left inguinal glands were removed, defatted, and dried with hot alcohol-ether. DNA and RNA were extracted by trichloroacetic acid and determined by diphenylamine and orcinol reactions, respectively. The procedures were essentially the same as described previously (5).

Histological Observation of Mammary Glands. The central part of the right inguinal gland was fixed in Bouin's solution, embedded in paraffin, sectioned at 6 μm, and stained with hematoxylin-eosin for histologic observation.

Endocrine Organ Weights. Anterior pituitary, adrenals, and ovaries were weighed at autopsy and adrenals and ovaries were further examined histologically.

Plasma Levels of Prolactin and Progesterone. Blood was drawn into the heparinized tube, centrifuged at 1000*g* for 20 min at 4°C, and stored at −20°C. Plasma prolactin level was determined by rat Nb_2 lymphoma cells bioassay (6). A highly purified mouse prolactin preparation (AFP-6476C) was used as the standard.

Plasma progesterone was extracted by petroleum ether, separated by Sephadex LH-20 column chromatography using the solvent of heptane:benzene: methanol = 85:10:5, and determined by radioimmunoassay using an antiserum generated to progesterone-11-BSA and [1,2,6,7-^{3}H]progesterone (7).

Statistics. All parameters were expressed in terms of mean ± SE and the statistical significance between groups was evaluated by Student's t test.

Correlation coefficients were calculated between the size of PDMT and the parameters for mammary gland function or plasma hormone levels in mice which experienced PDMT during pregnancy [PDMT(+) group].

Results

As shown in Figure 1, both weight and growth rate of pups on Day 12 of lactation, used as indices of lactational performance of mice, were significantly less in the PDMT(+) group than in the PDMT(−) group. Associated with this decrease in pup's growth, the DNA and RNA contents of the mammary gland were lower,

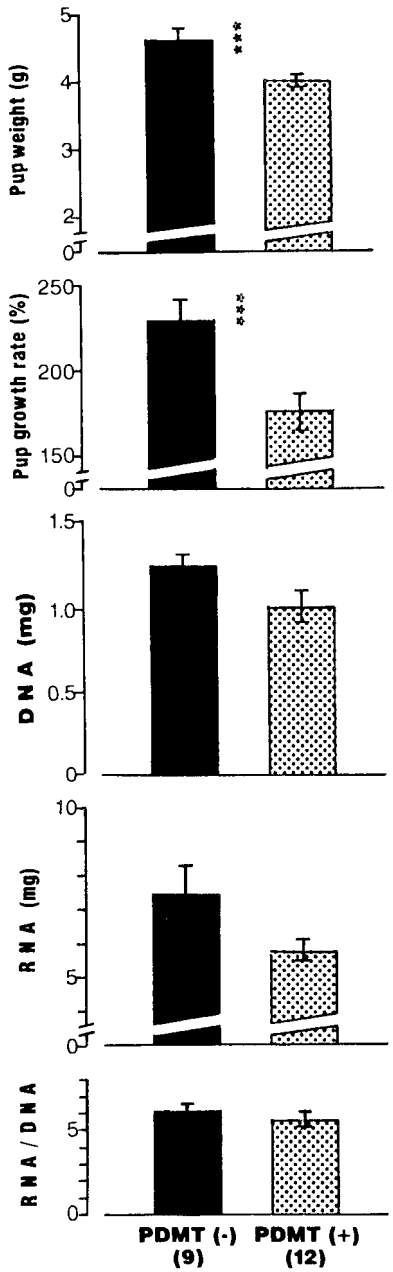

Figure 1. Mammary gland function in each group (mean ± SE). Only the data of mice which had 100% rearing rate—no loss of pups—were used. ***Significantly different at $P < 0.01$.

although the differences were not statistically significant. There was no difference in mammary RNA/DNA ratio between groups.

Reproductive parameters (Table I), plasma level of either prolactin or progesterone (Fig. 2), and endocrine organ weights (data not shown) were not different between PDMT(−) and PDMT(+) groups.

The morphology of the mammary glands, adrenals, and ovaries were also little affected by PDMT.

In addition, no significant correlations were obtained between the size of PDMT and parameters for mammary gland function or plasma hormone levels (Table II).

Discussion

This study shows that the pups' growth on Day 12 of lactation was significantly suppressed by PDMT, which were present only during pregnancy. The results were associated with a somewhat lower level of mammary DNA and RNA in mice with PDMT. Mammary RNA/DNA ratio, however, was little affected by PDMT. These findings indicate that suppressed lactation in mice with PDMT is principally attributable to the retarded growth of mammary parenchyma rather than the decreased function of mammary cells.

Prolactin and progesterone are the essential factors for the development and progression of PDMT as well as the growth and secretion of the glands (8–11). In this study, however, no difference was seen in plasma level of either prolactin or progesterone and in the morphology of the mammary glands, adrenals, and ovaries between mice which had PDMT and those which did not. These strongly suggest that retarded mammary gland growth is mostly due to the effects of PDMT during pregnancy.

No correlation was observed between the size of PDMT and parameters for mammary gland function or plasma hormone levels in this study, which suggests that the development of PDMT per se rather than their progression is of importance for retardation of mammary gland growth and lactational performance. However, further study is needed to drawn a clear conclusion.

PDMT appear only in GR/A and some other mouse strains originated in Europe such as RIII and BR6, but not in any other animal species. Thus, this animal model would be important for studies on the relationship between mammary tumors and lactation.

Table I. Reproduction in Each Group (Mean ± SE)

	PDMT (−)	PDMT (+)
No. of mice	11	14
Delivery interval (days)	25.4 ± 2.9	25.8 ± 2.7
Litter size	6.5 ± 0.5	6.6 ± 0.6
Still-birth rate (%)	0	0
Rate of still-born pups (%)	0	1.1
Rearing rate on Day 12 (%)	97.0 ± 3.0	92.9 ± 7.1
Mother weight (g)		
At parturition	28.0 ± 0.4	28.8 ± 0.4
Day 12	32.7 ± 0.6	32.9 ± 0.5

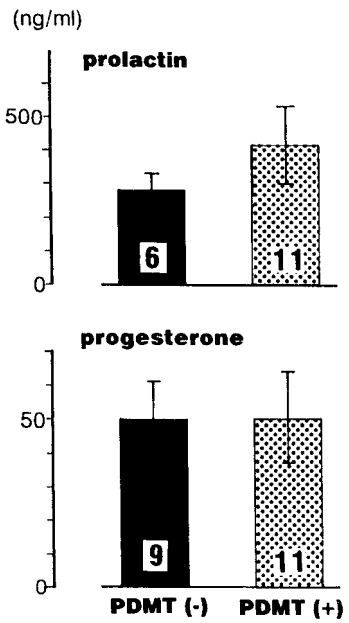

Figure 2. Plasma hormone levels in each group (mean ± SE).

Table II. Correlation Coefficients between Size of PDMT and Parameters for Mammary Gland Function or Plasma Hormone Level ($N = 12$)

Parameters	r
Size of PDMT[a]	
Pup weight	−0.24
Pup growth rate	−0.20
DNA	−0.40
RNA	−0.32
RNA/DNA	0.06
Plasma prolactin	0.40
Plasma progesterone	−0.22

[a] When mice developed more than two PDMT, sum of the sizes of all PDMT was used as the representative value of the individual. The number and size (mm) of PDMT per mouse were 1.3 ± 0.1 and 6.8 ± 1.0, respectivity.

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