MAMMARY GROWTH AND FUNCTION
AND PITUITARY PROLACTIN SECRETION IN
FEMALE NUDE MICE

By

Hiroshi Nagasawa and Reiko Yanai

ABSTRACT

Mammary structural growth in the wholemount preparation, content and synthesis of mammary DNA and RNA estimated by the incorporation of [³H]thymidine and [¹⁴C]uridine, pituitary and plasma levels of prolactin and weights and histological structures of some organs of female nude mice (nu/nu) were compared to those of the control (nu/+), with the same genetical background (BALB/c). Both at 3 months of age and on day 1 of lactation, the weights per 100 g body weight of adrenals, spleen and liver of nu/nu mice were significantly higher than those of nu/+ mice. Mammary growth stimulation by pituitary graft was much more marked in nu/nu mice than in nu/+ mice. Slight differences between groups were found in the pituitary and plasma levels of prolactin, in the histological structures of ovaries as well as of the adrenals and thyroids and in the pattern of oestrous cycles. On the other hand, the content and synthesis of mammary DNA at 3 months of age and content and synthesis of both DNA and RNA and RNA/DNA ratio on day 1 of lactation were significantly higher in nu/+ mice than in nu/nu mice. All findings suggest that thymus deficient immunosuppression has deleterious effects on mammary growth and function without its alteration in the secretion of prolactin and oestrogen and probably through its decrease in mammary responsiveness to mammotrophins.

Both immunity and endocrine function are two major factors for maintaining homoeostasis. However, there is a scanty evidence on the information of the interrelation between these factors. Most recently, the congenitally athymic

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nude mice have been available for the studies concerning this problem. Osawa et al. (1974) reported preliminarily on the functions of adrenals, thyroids, testes and pituitary (growth hormone) of nude mice. The importance of an immune mechanism on both human and experimental mammary tumours has recently been emphasized (Brennan 1976; Di Paola et al. 1974; Kuperman et al. 1975; Radov et al. 1976; Stutman & Herberman 1976). Thus, the study on the relation of a thymus deficient condition to mammary growth and function is particularly pertinent. In this paper, growth and function of mammary glands and pituitary prolactin secretion as well as oestrous cycles and histological structures of some organs of female nude mice were studied.

**MATERIALS AND METHODS**

**Animals**

Athymic nude mice (nu/nu) transferred to this laboratory from CLEA Japan Inc. or Institute of Medical Science, University of Tokyo and their offsprings were used. The control mice (nu/+), with the same background (BALB/c) were those from CLEA Japan Inc. and their offspring. They were kept 5 each in a teflon cage (12 × 30 × 15 cm) with wood shavings, covered by a filter cover (Iso-Cap®; SANKI Scientific Co. Ltd., Tokyo, Japan) and placed on Iso-rack® (SANKI Scientific Co. Ltd.) that can maintain animals under SPF conditions; fresh air filtered by double microfilters, with which particles of more than 0.3 μm in size were almost completely eliminated (99.9%), blows out gently and horizontally from the back wall of the rack. Drinking water, diet (CL-1; CLEA Japan Inc.), wood shavings, cages, water bottles and all the other utensils for feeding were sterilized. The isolated animal room was air-conditioned (25–26°C and 65–75% in relative humidity) and artificially illuminated (12 h light from 08:00 to 20:00 h).

**Body weight change**

The individual body weight in virgin females was measured every week between 4 and 11 weeks of age.

**Oestrous cycles and weights and histological structures of organs**

Vaginal smears were taken every morning (09:00–09:30 h) for about 30 days in 3 months old virgin mice. Some mice were bled on the late afternoon (about 17:00 h) of oestrus from the vena cava under light ether anaesthesia. After bleeding, they were killed by decapitation and the anterior pituitary, thyroids, adrenals, ovaries, spleen and liver were immediately removed and weighed. The anterior pituitary and plasma were frozen and kept at -20°C for assay of prolactin. Adrenals, thyroids and ovaries were fixed in Bouin’s solution, embedded in paraffin, then sectioned at 6 μm and stained with haematoxylin-eosin for histological observation.

On the morning of the next day of parturition (day 1 of lactation), primiparous mice were bled immediately after 30 min suckling following 1 h removal of the litter, the size of which was adjusted to 5 just before removal. The same organs as in the virgins except for the thyroids were removed and were similarly treated.
Growth and function of mammary gland

Three months old virgin mice were given intraperitoneal injections with 50 μCi [3H]thymidine (5 Ci/mmole; The Radiochemical Centre, Amersham, England) on the afternoon (about 15:00 h) of pro-oestrous/oestrus or dioestrous and killed 2 h later. The right third thoracic gland was used for the whole-mount evaluation. The degree of growth was rated from 1 to 7 in increments of 1. Bilateral inguinal glands were used for the in vivo incorporation of [3H]thymidine into mammary DNA as an index of the synthetic activity of DNA. The procedures were the same as described previously (Yanai & Nagasawa 1976). Since there was little difference between oestrous stages in the values in either group, the values at different stages were pooled in the section of Results.

In order to evaluate mammary responsiveness to pituitary mammotrophins, some mice at 3 months of age were isografted with 2 anterior pituitaries each under the right kidney capsule and killed 20 days later. Mammary growth in the whole-mount preparation and mammary DNA synthesis were compared with the non-treated controls of the same age in each group.

On day 1 of lactation, the right third thoracic and bilateral inguinal glands were used for the measurement of the in vitro incorporation of [3H]thymidine and [14C]-uridine into mammary DNA and RNA as the indices of the synthetic activity of DNA and RNA, respectively. Total contents of DNA and RNA were assayed using the left third thoracic glands. All procedures were similar to those described previously (Nagasawa & Yanai 1974).

Pituitary and plasma levels of prolactin

Pituitary and plasma levels of prolactin were assayed by homologous radioimmunoassay (Sinha et al. 1972) in 3 months old virgin mice at oestrous and in primiparous mice after 30 min suckling following 1 h litter removal on day 1 of lactation.

RESULTS

Body weight changes

The growth curve in each group is shown in Fig. 1. While the body weight was significantly greater in nu/+ virgin mice than in nu/nu mice at any age examined (P < 0.01), there was little difference between groups in the pattern of growth itself.

Oestrous cycles and weights and histological structures of organs

In both groups, oestrous cycles were rather irregular as usually seen in most mouse strains and no differences were observed between groups.

Organ weights expressed in terms of 100 g body weight in each group are illustrated in Table 1. Not only at the virginal stage but also on day 1 of lactation, the weights of the adrenals, spleen and liver were significantly greater in nu/nu mice than in nu/+ mice (P < 0.05 or 0.01). On the other hand, slight differences were found between groups in the weights of anterior pituitary, thyroids and ovaries. Histological structures of adrenals, thyroids and ovaries of nu/nu mice were similar to those of nu/+ mice.

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Growth curve of virgin females in each group (means ± SEM). Number of estimations is indicated in parentheses. The weight was significantly heavier in nu/+ mice than in nu/nu mice at any age examined ($P < 0.01$).

Litter size and average litter weight on day 1 of lactation were significantly higher in nu/+ mice than in nu/nu mice ($P < 0.01$).

**Growth and function of mammary glands**

The results of mammary growth are presented in Fig. 2. In 3 months old virgins, the content and synthesis of mammary DNA were significantly higher in nu/+ mice than in nu/nu mice ($P < 0.01$), while there was no difference between groups in the structural growth observed in the wholemount preparation, the glands consisting only of duct systems.

Grafting with 2 isologous pituitaries under the right kidney capsule resulted in the significant increase in mammary rating, content and synthesis of DNA in nu/nu mice and in rating and content of DNA in nu/+ mice when compared to the respective non-treated controls of the same age ($P < 0.01$). The rate of increase in the measures was more marked in nu/nu mice than in nu/+ mice. Pituitary grafting induced no significant difference between nu/nu mice and nu/+ mice in any measure examined.

On day 1 of lactation, content and synthesis of both DNA and RNA and RNA/DNA ratio were significantly higher and the $^{14}\text{C}/^{3}\text{H}$ ratio was lower in nu/+ mice than in nu/nu mice (Figs. 2 and 3) ($P < 0.01$).

The ratios of SEM against means in the synthesis of DNA and RNA of nu/nu mice on day 1 of lactation were much higher than those of nu/+ mice [0.21...
Table 1.
Organ weight per 100 g body weight in each group (means ± SEM).

<table>
<thead>
<tr>
<th>Stage</th>
<th>Group</th>
<th>No. of mice</th>
<th>Litter size</th>
<th>Litter weight (5 pups) (g)</th>
<th>Body weight (g)</th>
<th>Anterior pituitary (mg)</th>
<th>Thyroids (mg)</th>
<th>Adrenals (mg)</th>
<th>Ovaries (mg)</th>
<th>Spleen (mg)</th>
<th>Liver (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Virgins</td>
<td>nu/+</td>
<td>8</td>
<td>-</td>
<td>-</td>
<td>24.9 ± 0.6</td>
<td>6.2 ± 0.3</td>
<td>14.0 ± 1.0</td>
<td>29.6 ± 1.4</td>
<td>42.0 ± 1.5</td>
<td>391 ± 16</td>
<td>5.5 ± 0.2</td>
</tr>
<tr>
<td>(3 months)</td>
<td>nu/nu</td>
<td>9</td>
<td>-</td>
<td>-</td>
<td>21.8 ± 0.6</td>
<td>6.7 ± 0.3</td>
<td>12.6 ± 0.6</td>
<td>37.5 ± 0.9</td>
<td>44.2 ± 3.0</td>
<td>573 ± 30</td>
<td>6.3 ± 0.1</td>
</tr>
<tr>
<td>Primipara</td>
<td>nu/+</td>
<td>10</td>
<td>8.3 ± 0.5</td>
<td>9.0 ± 0.1</td>
<td>33.4 ± 0.7</td>
<td>7.1 ± 0.3</td>
<td>-</td>
<td>22.4 ± 1.0</td>
<td>51.7 ± 1.6</td>
<td>484 ± 24</td>
<td>6.7 ± 0.1</td>
</tr>
<tr>
<td>(day 1 of lactation)</td>
<td>nu/nu</td>
<td>10</td>
<td>6.4 ± 0.3</td>
<td>7.1 ± 0.1</td>
<td>25.8 ± 0.3</td>
<td>6.3 ± 0.3</td>
<td>-</td>
<td>30.8 ± 1.8</td>
<td>56.2 ± 2.1</td>
<td>728 ± 31</td>
<td>7.2 ± 0.1</td>
</tr>
</tbody>
</table>

P < 0.01 < 0.01 < 0.01 > 0.05 < 0.01 > 0.05 < 0.01 < 0.01
Mammary rating, content and synthesis of mammary DNA at 3 months of age and on day 1 of lactation in each group (means ± SEM). Number of estimations is indicated in parentheses. Control: Non-treated, 2AP: Grafted with 2 isologous pituitaries each. Differences between nu/+ mice and nu/nu mice in content and synthesis of DNA were statistically significant both at 3 months of age and on day 1 of lactation (P < 0.01). Pituitary grafting increased significantly all measures in both groups (P < 0.01) except for DNA synthesis in nu/+ mice.

(200/959) and 0.12 (440/3747) for DNA synthesis (Fig. 2) and 0.23 (182/790) and 0.08 (132/1813) for RNA synthesis (Fig. 3) in nu/nu mice and nu/+ mice, respectively.

**Pituitary and plasma levels of prolactin**

As shown in Table 2, either at oestrus of virginal stage or on day 1 of lactation, there were no significant differences between groups regarding the pituitary and plasma levels of prolactin except for the total pituitary content on day 1 of lactation, which was significantly higher in nu/+ mice than in nu/nu mice (P < 0.01).
Content and synthesis of mammary RNA on day 1 of lactation in each group (means ± sem). Number of estimations is indicated in parentheses. Nu/+ mice were significantly different from nu/nu mice in any parameter ($P < 0.01$).

**Table 2.**

Pituitary and plasma levels of prolactin in each group (means ± sem).

<table>
<thead>
<tr>
<th>Stage</th>
<th>Group</th>
<th>No. of estimations</th>
<th>Pituitary prolactin</th>
<th>Plasma prolactin (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>µg/µg pituitary</td>
<td>µg/mg pituitary</td>
</tr>
<tr>
<td>Virgins (3 months, at oestrus)</td>
<td>nu/+</td>
<td>10</td>
<td>4.91 ± 0.30</td>
<td>3.08 ± 0.20</td>
</tr>
<tr>
<td></td>
<td>nu/nu</td>
<td>9</td>
<td>4.58 ± 0.33</td>
<td>3.17 ± 0.15</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>&gt; 0.05</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Primipara (day 1 of lactation, after suckling)</td>
<td>nu/+</td>
<td>10</td>
<td>3.97 ± 0.29</td>
<td>1.71 ± 0.16</td>
</tr>
<tr>
<td></td>
<td>nu/nu</td>
<td>10</td>
<td>2.98 ± 0.14</td>
<td>1.88 ± 0.12</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>&lt; 0.01</td>
<td>&gt; 0.05</td>
</tr>
</tbody>
</table>
DISCUSSION

The present study shows that there were slight differences between the groups in the pituitary and plasma levels of prolactin and that the weight and histological structure of the ovaries and the pattern of the oestrous cycle of nu/nu mice were quite similar to those of nu/+ mice. These data indicate that the secretion of prolactin and possibly oestrogen of nu/nu mice is not much different from that of nu/+ mice. Nevertheless, nu/nu mice were inferior to nu/+ mice in almost all the determinations as the indices of mammary growth and function. These findings postulate that thymus mediated immunosuppression induces stagnancy in mammary growth and function through its decrease in the mammary responsiveness to mammotrophins, whereas it has little effects on the secretion of the hormones.

A more marked increase by pituitary grafting in measures as the indices of mammary growth in nu/nu mice would mainly be attributable to the higher secretion of prolactin from the grafts of nu/nu mice than from those of nu/+ mice, which resulted from less resistance to the grafts in the former than in the latter animals.

Larger variations in the values of nucleic acid synthesis in nu/nu mice than in nu/+ mice on day 1 of lactation indicate that immunosuppression could also induce the decrease in the dynamic stability of mammary growth and function, despite the utmost compensation of animals for athymic homoeostatic imbalance which is reflected by the increase in the relative weights of adrenals, spleen and liver.

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REFERENCES


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