Morphometric Study on the Fetal Thyroid Gland in the Nude Mouse (BALB/cAnNCrj-nu/nu)

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Abstract: Fetal thyroid glands of athymic nude mice (BALB/cAnNCrj-nu/nu) were examined morphometrically on day 18 of gestation. Compared to euthymic litter mate controls (BALB/cAnNCrj-nu/+), both the cell height and diameter of thyroid follicles were significantly smaller; fewer well-developed follicles were found in the peripheral region of the thyroid; the body weight and total volume of the thyroid gland were also smaller in nude mice. These results suggest underdevelopment of the fetal thyroid gland of the athymic nude mouse.

Key words: BALB/c-nu/nu, fetus, thyroid gland

Nude mouse (nu/nu) lacks the thymus gland and shows dysfunction of the cellular immune system [13, 14, 24].

In addition, it has long been claimed that the function of endocrine organs including the ovary, testis, thyroid, and adrenal glands was impaired in nude mice [3, 7, 15, 20, 21]. According to Ohsawa *et al.* [12], however, there are no significant differences between euthymic mouse (BALB/c or BALB/c-nu/+) and athymic nude mouse (BALB/c-nu/nu) in the function of endocrine gland, if nude mice with known genetic and micro-biological backgrounds have been kept in SPF or germ-free conditions.

Recently, some authors reported that the thymus secretes hormone-like substances including thymosin and thymopentin to regulate or stimulate the thyroid, testis

and adrenal glands directly or indirectly via the hypothalamo-hypophyseal axis [2, 4, 8, 9, 17–19, 23]. In the present study, therefore, in an attempt to reassess the function of endocrine organs in nude mice, we compared the thyroid gland morphometrically in athymic fetal nude mice and euthymic litter mate mice.

Animals: Male BALB/cAnNCrj-nu/nu and female BALB/cAnNCrj-nu/+ mice were purchased from Charles River Co., Kanagawa, Japan. The animals were kept in a room with a barrier system. The room was air-conditioned at a temperature of $25 \pm 1^\circ\text{C}$ and humidity of $65 \pm 5\%$, and lighted for 14 hr per day, from 6:00 to 20:00. Each animal was kept in a polycarbonate plastic cage (CL-0104-2; $215 \times 320 \times 130$ mm; CLEA Japan Inc., Tokyo, Japan) with wood shavings on the floor. The cage was covered with a filter cover

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(Iso-Cap®; SANKI Scientific Co., Tokyo, Japan) and placed in a stainless steel isolator (EBAC-L, CLEA Japan Inc.). Animals were fed *ad libitum* with an irradiated bacteria-free laboratory ration (mouse and rat chow; CRF-1, Charles River Co.) and autoclaved water. Wood shavings, cages, water bottles and the other devices were used only after being autoclaved.

Each female was mated with a male, and was checked for the presence of a vaginal plug every morning. The day when a plug was observed in the vagina was counted as day 0 of gestation. Three pregnant mothers were sacrificed by decapitation under sodium pentobarbital anesthesia on day 18 of gestation to remove fetuses. The fetuses were weighed and then sacrificed by decapitation under hypothermic anesthesia with ice cubes. The decapitated fetuses were thoracostomized to determine whether the thymus was present or not. The thyroid was immediately removed together with the larynx and trachea under a binocular operating microscope (OCS-PS, Olympus Optical Co., Tokyo, Japan) and fixed in Bouin's fluid.

Microscopic observation: Fixed thyroid specimens were dehydrated through a graded series of ethanol, embedded in paraffin, sectioned serially at 6 μm and stained with hematoxylin-eosin. Morphometric analysis was performed on the left thyroid by the means of an Olympus-Avio Color Image Analyzer SP500 (Olympus Optical Co., Tokyo, Japan).

The thyroid volume was determined from the following formulas:

$\text{Si (actual area of section)} = \text{area of traced outline (cm}^2\text{)}/\text{linear magnification}^2$.

$\text{Volume (mm}^3\text{)} = \Sigma \text{Si (cm}^2\text{)} \times \text{distance between every tenth section (cm)}$.

The average diameter of follicles was determined for each thyroid by the measuring diameters of a total of 28 well-developed large follicles; four in the largest

section and four each in three of every five sections before and after the largest one.

The average cell height was determined similarly in a total of 35 follicular cells for each thyroid gland, each five cell was selected at random in the same 7 sections used for the determination of follicular diameter.

Statistical analysis: The data were expressed as the mean $\pm$ standard error mean (SEM) and analyzed by Student's *t*-test. The inter-group difference was considered as statistically significant when the *p* value was less than 0.05.

Gravimetric data for fetal nude mice are summarized in Table 1. The body weight and the volume of the left thyroid lobe were slightly but not significantly smaller in the athymic mice than in the euthymic litter mate mice. The follicular diameters and follicular cell heights of the left thyroid lobe were significantly smaller in the athymic group than in the euthymic group. The number of well-developed follicles identified in the periphery of the gland was smaller in the athymic group than in the euthymic group (Figs. 1 and 2).

Ohsawa *et al.* [12] reported that athymic nude mice kept in the SPF environment showed signs of the same normal endocrine functions as seen in euthymic mice. In the present study, however, the results showed underdevelopment of the thyroid gland in the fetal athymic nude mice, although we took fetuses from euthymic mothers reared under SPF conditions.

Ohsawa *et al.* [12] also recognized that body growth was slower in nude mice than in euthymic mice in the neonatal period before weaning. The lower rate of body growth in athymic nude mice was also reported by other researchers [6, 16], suggesting the possibility of a hypofunctional state of the thyroid or pituitary gland, both of which secrete hormones with a very important role in body growth and metabolism.

Aleshin *et al.* [1] noted that induced thymus inflam-

Table 1. Quantitative data on fetal thyroid glands in *nu/nu* and *nu/+* mice

Age (days)	No. of animals	Body weight (g)	Left thyroid lobe volume (mm ³ $\times 10^{-3}$)	Left thyroid lobe volume/100 g body weight (mm ³ $\times 10^{-3}$)	Thyroid follicular diameter (μm)	Thyroid follicular cell height (μm)
18	<i>nu/nu</i> 6 (3)*	0.95 $\pm$ 0.02**	49.2 $\pm$ 3.90	51.9 $\pm$ 3.92	18.62 $\pm$ 0.48 ^{a)}	6.00 $\pm$ 0.15 ^{a)}
18	<i>nu/+</i> 6 (3)	1.00 $\pm$ 0.03	57.6 $\pm$ 5.24	58.3 $\pm$ 5.06	20.68 $\pm$ 0.40	6.72 $\pm$ 0.06

* = No. of litters in parenthesis. ** = mean $\pm$ SEM. ^{a)} = Significantly different from fetal *nu/+* mice.

Fig. 1. Thyroid gland of a fetal euthymic litter mate nude mouse on day 18 of gestation. Well-developed follicles are observable in the thyroid gland (see arrows). $\times 400$.

Fig. 2. Thyroid gland of a fetal athymic nude mouse on day 18 of gestation. There are few well-developed follicles in the thyroid gland (see arrows). $\times 400$.

mation with melted paraffin droplets or the administration of thymic-extract (thymosin) caused an increase in thyroid weight and follicular cell height in rabbits and guinea pigs. Milenkovic and McCann [11] reported that another thymic-extract, thymosin $\alpha 1$, could induce the release of thyrotropin (TSH) from the pituitary gland *in vitro*. The other thymic-extract components such as thymosin $\beta 4$ and $\beta 5$ and thymopentin were also reported in several studies to have gonadotropin-releasing (rat, *in vitro*), corticotropin-releasing (human and primates, *in vivo*), and growth hormone-releasing (human, *in vivo* and rat, *in vitro*) activity [2, 5, 8, 9, 17, 22, 23]. Taking these reports into consideration, the underdevelopment of the thyroid gland of athymic nude mice which we observed in this morphometrical study may be caused by the absence of the thymus.

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