Tohoku J. Exp. Med., 1997, **182**, 309-318

Genetically Determined Thymus Enlargement in the Early Life in BUF/Mna Rats

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Department of Pathology, Nagoya University School of Medicine, Nagoya 466, [1]*Aichi Women's College, Nisshin 470-01,* [2]*Nagoya City Public Health Research Institute, Nagoya 467,* [3]*Central Institute for Experimental Animals, Kawasaki 216, and* [4]*Department of Pathology, Fujita Health University School of Medicine, Toyoake 470-11*

Saito, M., Amo, H., Murakumo, Y., Kojima, A., Katoh, H., Hiromatsu, T. and Matsuyama, M. *Genetically Determined Thymus Enlargement in the Early Life in BUF/Mna Rats.* Tohoku J. Exp. Med., 1997, **182** (4), 309-318 —— BUF/Mna (B) rat is a mutated strain, having much larger thymus than WKY/NCrj (W), ACI/NMs (A), and F344 (F) rats throughout their life-span. Rats of the latter 3 strains have normal sized thymuses, being less than 5.3 in the thymus to body weight ratio (mg/g), when they were killed at 6 weeks of age. Genetic segregation of large thymus size in the B strain at 6 weeks of age was studied by crossing B rats with W, A or F rats. All of 3 types of the F1 hybrid rats between the B strain and the other strains showed intermediate thymus ratios between those of both parental strains. In F2 rats between the B and W strains, the distribution of thymus ratios showed about 5 different peaks. These findings might indicate that two polymeric autosomal loci, thymus enlargement-1 (*Ten-1*) and thymus enlargement-2 (*Ten-2*), can enlarge the thymus size in B rats. Histometrically, whole thymus and cortex areas of the B rats were 2-5 times larger than the W rats during 6-12 weeks of age, but medulla areas were slightly different between the strains, showing that larger thymuses in B rats were mainly due to the enlarged cortex areas. —— BUF/Mna rats; genetic analysis; *Ten-1* and *Ten-2*; thymus size enlargement; cortex area/medulla area ratio

Slight differences among inbred rat strains in thymus weight have been observed. In the course of a study on the development of thymoma, we noticed, however, that the thymuses of BUF/Mna (B) rats were much larger than those of ACI/NMs (A) rats at all ages (Matsuyama et al. 1988). It has recently been found

Received February 1, 1997; revision accepted for publication June 20, 1997.

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that this extraordinary large thymus of B rat in the young period is regulated by two mutated genes, thymus enlargement-1 and thymus enlargement-2 (*Ten-1* and *Ten-2*, Murakumo et al. 1993, 1996). In the mouse, two genes were also reported to control the thymus size in the young period (Peleg and Nesbitt 1984). Remarkable increases in thymus weight has been reported in mice bearing an inheritable defect in the androgen receptor protein (testicular feminization: *Tfm/Y*) (Olsen and Kovacs 1989). Furthermore, thymic hyperplasia was developed in transgenic mice harboring a growth hormone-releasing factor (GRF) promoter fused to SV40 large T antigen (Botteri et al. 1987). These findings prompted us to perform genetic studies, using large numbers of rats of various mating groups, to confirm the presence of the *Ten-1* and *Ten-2* loci that enlarge the thymus size in B rats. It was also examined histometrically which parts of the thymus, the cortex or medulla, enlarged in B rats.

Materials and Methods

The origin and biological properties of inbred rats of the B and A strains, maintained in our laboratory, were described earlier (Matsuyama et al. 1972, 1986). Inbred WKY/NCrj (W) and F344 (F) rats were originally supplied by Charles River Japan, Inc., in 1985, and by Dr. J. Kitoh, Institute for Laboratory Animal Research, Nagoya University School of Medicine, in 1990, respectively.

Rats of these 4 strains were raised by brother and sister matings, and rats of F1, F2 or backcross (BC) generations were raised by crossing either B with W, B with A, or B with F. All rats were weaned at 5 weeks of age and given a pellet diet, CMF (Oriental Yeast Co., Tokyo), and tap water freely.

To know the suitable time to differentiate the thymus size among the strains, rats of the 4 strains were killed under ether anesthesia at 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12 weeks after birth. For genetic analyses F1, F2 and BC rats were killed at 6 weeks of age. The body and thymus weights were determined and calculated the ratios of thymus weights to body weights (mg/g).

The thymuses were fixed in 10% formalin and histologic sections were cut at the level of largest cut surface of the organ, and stained with hematoxylin and eosin. The areas of the cortices, medullae, and whole thymuses were measured by an image analyzer, Vidas (Kontron Elektronik, Eching, Munich), and cortex area/whole thymus area ratios and cortex area/medulla area ratios were calculated.

The thymus ratios, whole thymus areas, cortex areas, medulla areas, cortex area/whole thymus area ratios (%), and cortex area/medulla area ratios were analyzed by Student's *t*-test.

Results

Thymus ratios of B, W, A and F rats in the suckling and young adult periods

Thymus ratios of female rats of these strains during suckling and young adult periods were shown in Fig. 1. The curve of the ratios of B rats was highest among

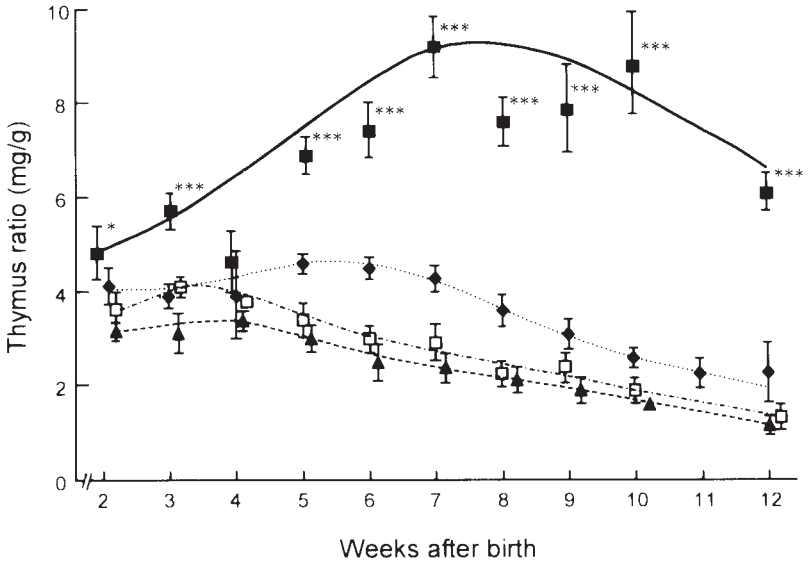

Fig. 1. Thymus ratio curves of female BUF/Mna (B) ■, ACI/NMs (A) ◆, F344 (F) □, and WKY/NCrj (W) ▲ rats in the suckling and young adult periods. The bars represent standard deviations. $^{*}p<0.05$ and $^{***}p<0.001$, compared to those of A, F and W rats.

the 4 strains and showed an increasing trend until the 7th week. Thereafter, the ratios lowered slowly, showing the physiological involution. The curve of the A strain was much lower than that of the B strain, declining from the 5th week (Fig. 1). The curves of the F and W strains were lowest, showing declining trends from the 3rd and 4th week, respectively. The thymus ratios of male rats of these strains showed slightly lower but similar curves to those of female rats (data not shown). From these results, 6 weeks of age was chosen as a suitable time to differentiate the thymus size among the strains.

Thymus ratios of rats of the B, W, A and F strains, and of their F1, F2 and/or BC at 6 weeks of age

The actual thymus weights and thymus ratios examined at 6 weeks of age were statistically larger in the B strain than in the W, A, and F strains (Table 1 and Figs. 2 and 3). The mean actual thymus weights of the B rats were 3.2 and 3.1 times larger than the W rats in females and males, respectively. Similarly, the thymus ratios were 2.8 and 2.7 times larger in the female and male B rats, respectively. No sex difference was found in the thymus ratios, except B, (B×BAF1) BC, and (B×ABF1) BC rats. There was no difference in the ratios between reciprocal BWF1 and WBF1 rats, and between reciprocal BAF1 and ABF1 rats, showing no maternal effect (Table 1). All 3 types of the F1 hybrids between the B strain and the W, A or F strain had intermediate thymus ratios, showing one peak, between those of the parental strains (Table 1 and Figs. 2 and 3). In BWF2 and WBF2 rats, about 5 peaks were observable (Fig. 2). Furthermore, about 3 peaks were observable in the (BWF1×W) BC, (WBF1×W) BC

TABLE 1. *Thymus weights and thymus ratios of BUF/Mna (B), WKY/NCrj (W), ACI/NMs (A), and F344 (F) rats, and of their hybrid rats at 6 weeks of age*

Strain and mating group	Sex	Number of rats	Body weight (g)	Thymus weight (mg)	Thymus ratio (mg/g)
B	F	47	108±10	803±110	7.4±0.7
	M	34	130±15	845±153	6.5±0.5
W	F	67	99±10	251± 31	2.6±0.3***
	M	61	115±13	277± 30	2.4±0.2***
A	F	52	106±10	478± 80	4.5±0.6***
	M	54	132±14	521± 82	4.0±0.5***
F	F	32	105±11	307± 36	3.0±0.2***
	M	21	127±15	328± 42	2.6±0.2***
BWF1	F	20	125±10	549± 37	4.4±0.3***
	M	30	147±14	631± 55	4.3±0.3***
WBF1	F	63	111±15	446± 68	4.2±0.4***
	M	53	135±20	560± 72	4.2±0.3***
BWF2	F	20	113±14	487±157	4.3±1.1***
	M	17	138±12	580±125	4.2±0.7***
WBF2	F	68	105±12	412±102	3.9±0.8***
	M	60	123±17	471± 85	3.9±0.7***
(B×BWF1) BC	F	65	118±15	693±156	5.9±1.0***
	M	37	136±20	749±167	5.5±0.7***
(B×WBF1) BC	F	11	118± 8	651± 98	5.5±0.7***
	M	9	143±16	764±132	5.3±0.8***
(BWF1×B) BC	F	72	121±11	709±149	5.9±1.0***
	M	74	141±15	773±139	5.5±0.8***
(WBF1×B) BC	F	81	118±14	670±146	5.9±1.1***
	M	94	138±17	767±164	5.5±1.0***
(BWF1×W) BC	F	43	108±10	350± 54	3.2±0.4***
	M	36	127±12	408± 60	3.2±0.4***
(WBF1×W) BC	F	15	115±13	349± 43	3.0±0.3***
	M	18	145±25	418± 68	2.9±0.4***
(W×BWF1) BC	F	33	105±11	324± 54	3.1±0.4***
	M	41	124±12	393± 60	3.2±0.4***
(W×WBF1) BC	F	12	92± 7	350± 52	3.8±0.4***
	M	16	116±11	368± 59	3.2±0.4***
BAF1	F	50	120±10	773± 93	6.4±0.4***
	M	32	143±17	855±121	6.0±0.4*
ABF1	F	26	134±10	826± 82	6.2±0.3***
	M	19	154±14	919± 93	6.0±0.4***
ABF2	F	46	117±10	715±123	6.1±0.8***
	M	39	151±12	815±145	5.4±0.8***

TABLE 1. Continued

Strain and mating group	Sex	Number of rats	Body weight (g)	Thymus weight (mg)	Thymus ratio (mg/g)
(B×BAF1) BC	F	48	122± 9	918±132	7.5±1.0
	M	54	146±12	952±148	6.5±0.9
(B×ABF1) BC	F	25	122±12	902±183	7.4±1.1
	M	20	150±21	967±243	6.4±1.0
(ABF1×B) BC	F	33	123±14	911±179	7.4±0.9
	M	27	145±19	985±199	6.8±0.8
(ABF1×A) BC	F	25	118± 8	570± 71	4.8±0.4***
	M	29	146±17	671± 88	4.6±0.4***
(A×BAF1) BC	F	18	105±15	521±100	5.0±0.5***
	M	16	124±17	602±122	4.8±0.5***
(A×ABF1) BC	F	29	121± 7	606± 83	5.0±0.6***
	M	34	142±19	686± 99	4.8±0.5***
FBF1	F	15	112± 9	572± 65	5.1±0.3***
	M	27	136±19	619± 89	4.6±0.4***

Each value is expressed as the mean±S.D.

Statistically significant at $*p<0.05$, or $***p<0.001$, when compared to those of BUF/Mna rats.

and (W×BWF1) BC rats (Fig. 2). The similar patterns were also found in F2 and BC rats between the B and A strains (Fig. 3). These findings might denote that the large thymus size in B rats results from the co-operating effects of 2 polymeric dominant genes, *Ten-1* and *Ten-2*, which can enlarge thymus size.

Whole thymus, cortex, and medulla areas, cortex area/whole thymus area ratios, and cortex area/medulla area ratios of rats of the B, W and A strains

Whole thymus and cortex areas were much larger in B rats than rats of the other 2 strains, when they were older than 6 weeks, being 2-5 times, as shown in Figs. 4 and 5. Medulla areas were 1.5-2 times larger in B rats (Fig. 5). Consequently, cortex area/whole thymus area ratios (data not shown) and cortex area/medulla area ratios were much larger in B rats (Fig. 5). Thus, the conclusion that the enlargement of the whole thymus in B rats results mainly from the enlargement of the cortex can be drawn.

DISCUSSION

The thymus size is regulated by many extrathymic and intrathymic factors in normal rats. However, it was well established that B rats had abnormally larger thymuses than A rats (Matsuyama et al. 1988). It was also found that absolute numbers of thymic nurse cells per whole thymus were extremely higher in B rats

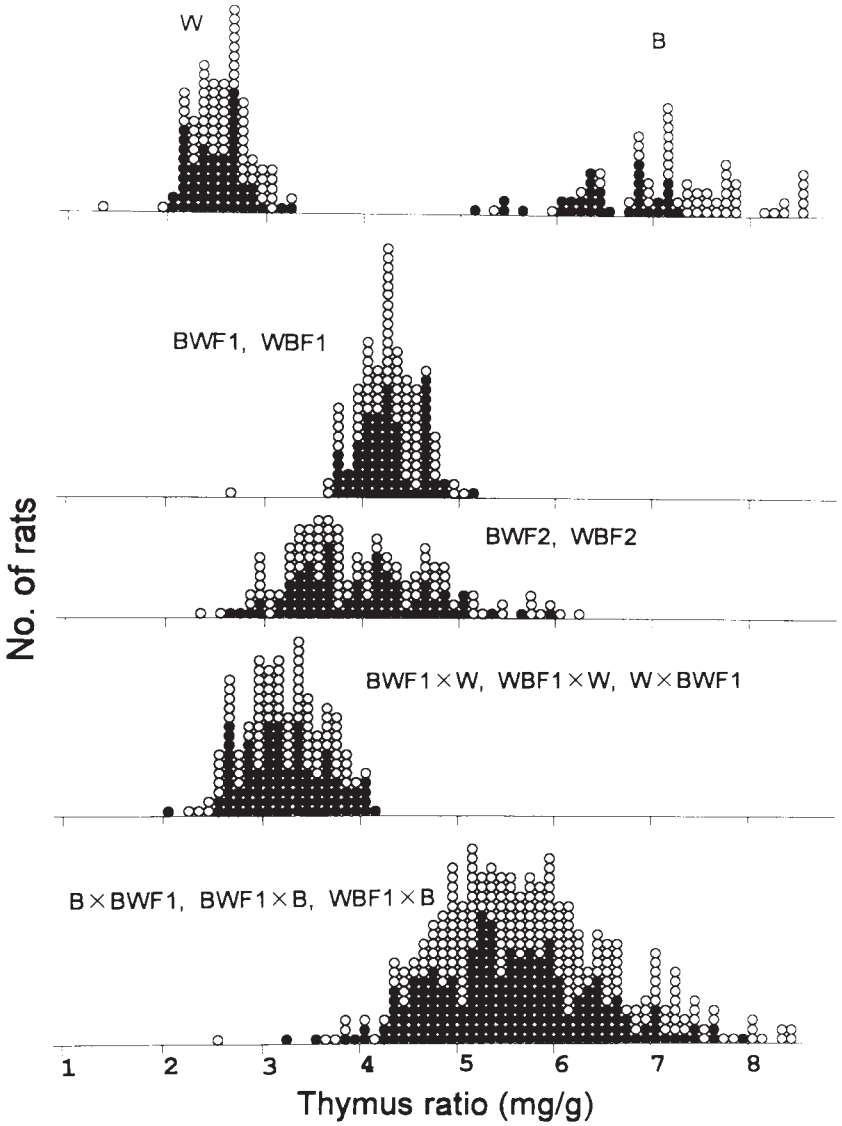

Fig. 2. Thymus ratio distributions in rats of the B and W strains and of their hybrid and BC generations at 6 weeks of age. Each circle shows 1 rat: female ○, male ●.

than those of control DA rats at all ages (Ezaki et al. 1991), resulting in the large increment of thymic lymphocytes. Our recent linkage study between genotypes of rat microsatellite or restriction fragment length-polymorphism markers and thymus sizes have shown that two genetic loci, *Ten-1* and *Ten-2*, which can enlarge thymus sizes in B rats, reside on chromosomes 1 (Murakumo et al. 1993) and 13 (Murakumo et al. 1996), respectively. The peaks in the distribution of thymus ratios in the F2 and BC rats obtained in the present study (Figs. 2 and 3) are not so distinct, but it is reasonable to consider that there are about 5 peaks in the F2 rats, if one assumes that many environmental factors affect the thymus size. These findings might mean that the abnormally large thymus of B rats is mainly regulated by 2 major polymeric genes, confirming the results of the previous molecular examinations (Murakumo et al. 1993, 1996). It is noteworthy that the large thymus size in the postnatal period (0–23 days) of C57BL/6J and ARK/J

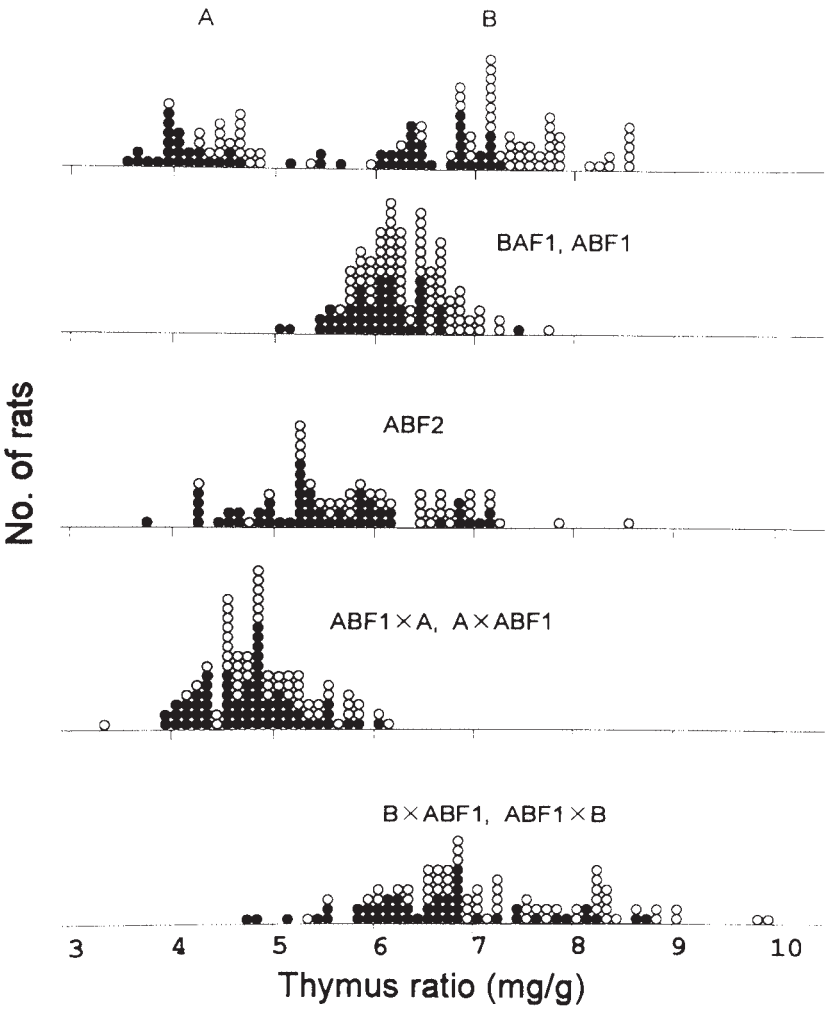

Fig. 3. Thymus ratio distributions in rats of the B and A strains and of their hybrid and BC generations at 6 weeks of age. Each circle shows 1 rat: female ○, male ●.

Fig. 4. Histological sections of thymuses of a B rat (on the left) and of a W rat (on the right). ×4.0.

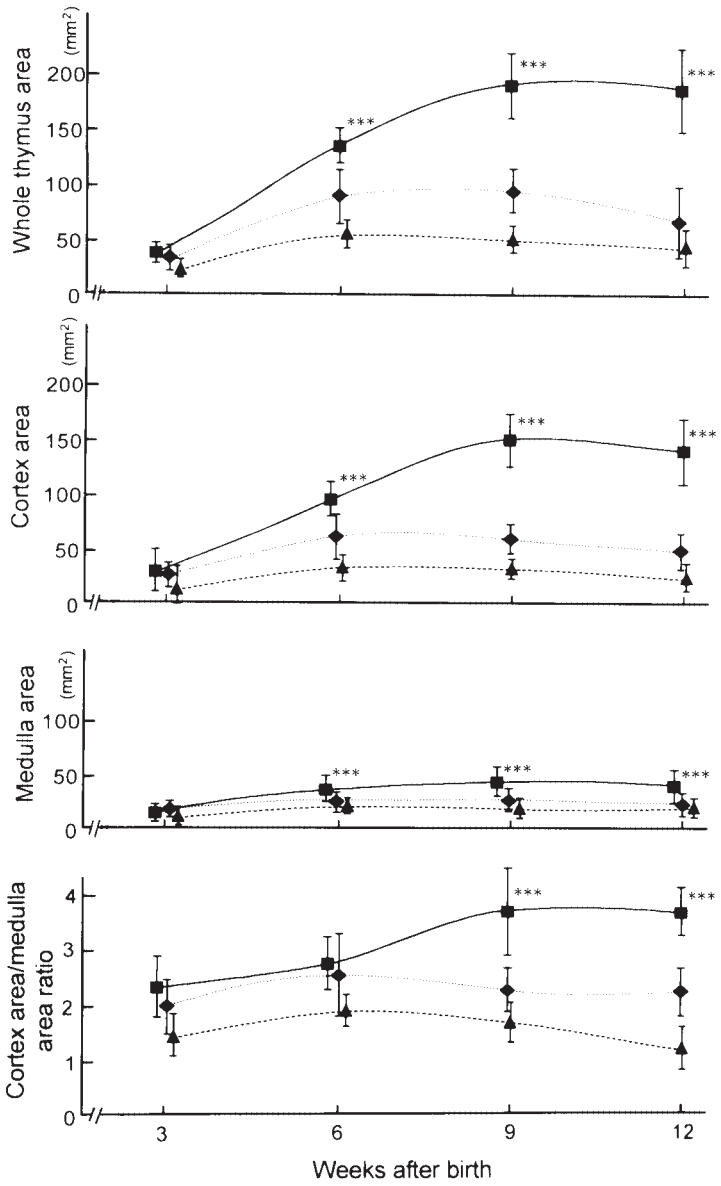

Fig. 5. Results of histometric analyses on whole thymus areas, cortex areas, medulla areas, and cortex area/medulla area ratios in B (■), A (◆), and W (▲) rats of 3, 6, 9 and 12 weeks of age. *** $p<0.001$, compared to those of A and W rats.

mice appeared to be controlled by two genes (Peleg and Nesbitt 1984). These genes might encode unknown enzymes and/or transcriptional factors related to the proliferation of cortical epithelial cells, which induce the proliferation of thymic lymphocytes (Kasai and Hirokawa 1991; Villa-Verde et al. 1991; Meilin et al. 1992; Ropke and Elbroend 1992; Takeuchi et al. 1995).

The thymus ratios of F1 rats of three types of the crosses were intermediate between those of the parental strains. These findings might be explained by the gene dosage and penetrance effects of *Ten-1* and *Ten-2* under the environmental conditions. The fact that BWF2 and WBF2 rats did not yield offspring with large thymuses comparable to those of B rats might also be implicated by the low gene dosage effects or action of suppressive genes from W rats. A rats may lack

such suppressive genes, since ABF2 rats gave a few offspring with large thymuses comparable to those of B rats. A few rats of (B×ABF1) and (ABF1×B) BC generations showed larger thymus ratio than the B rats. The reason of this overshoot in the thymus ratio might be due to the deletion of a suppressive gene located on chromosome 3, which has recently been found in BC rats between the B and MITE strains (Sharma et al. 1997).

Our previous studies showed that thymoma susceptibility was determined principally by a single autosomal dominant gene, *Tsr-1* (formerly *Tbm-1*), between the B and A strains (Matsuyama et al. 1986), and that thymoma nodules were developed in the background of the hyperplastic thymuses (Matsuyama et al. 1988). This development of thymomas in B rats is due to an intrinsic thymic epithelial abnormality (Taguchi et al. 1992). Therefore, a study to decide whether *Ten-1* or *Ten-2* is allelic to *Tsr-1* is under way.

It is interesting to note that transgenic mice harbored the GRF promoter fused to the SV40 large T antigen (TAG) developed thymic hyperplasia (Botteri et al. 1987). In these mice, expression of SV40 TAG is restricted to the thymic epithelial cells and proportions of 4 major T-cell populations (Ly-2$^+$ L3T4$^+$, Ly-2$^-$ L3T4$^+$, Ly-2$^+$ L3T4$^-$ and Ly-2$^-$ L3T4$^-$) were normal.

Acknowledgments

We are thankful to Mr. J. Aoki and Miss M. Kozuka for their technical assistance, and Mr. L.W. Roberts, Aichi Women's College, for critical reading of this manuscript. This study was supported by a Grant-in-Aid for Cancer Research from the Ministry of Education, Science, Sports and Culture of Japan, and by a grant from Imanaga Medical Foundation.

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