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Nodular Development of Spontaneous Epithelial Thymoma in (ACI/NMs × BUF/Mna)F1 Rats

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The BUF/Mna strain is a high thymoma line of rats, and virtually all rats develop overt thymomas by the age of 40 weeks. To reveal the early morphologic changes in this thymomagenesis, thymuses and thymomas were studied in (ACI/NMs × BUF/Mna)F1 (ABF1) rats, which inherit a thymoma susceptibility gene (*Tsr-1*) from the BUF/Mna strain. At 50 weeks of age, 18% of ABF1 rats had developed medium to large thymomas, 54% had just began to develop multiple, small round nodules in their involuted thymuses, and the remaining 29% had involuted thymus only. The nodules were, microscopically, composed of cortex-like tissues with a starry-sky pattern, showing a quite similar structure to that of the large macroscopic thymomas of predominantly lymphocytic type seen in 104-week-old ABF1 or BUF-Mna rats. Thus, the nodule was actually a small thymoma. In fact, their epithelial cells often had larger atypical nuclei than those in the adjacent involuted thymus cortex. At 104 weeks of age, the incidences of the medium to large thymomas and the small thymoma nodules in ABF1 rats were 64 and 19%, respectively. These results suggest that the thymoma of ABF1 rats occurs initially as multiple small nodules which develop further into medium to large overt thymomas as a result of growth and fusion.

Key words: Nodular development — Spontaneous epithelial thymoma — (ACI/NMs × BUF/Mna)F1 rats

The BUF/Mna strain has been established as a high thymoma line of rats.[1] The rat thymoma, which has quite similar microscopic features to those of human thymoma, develops spontaneously in this inbred strain at an incidence of nearly 100%. Crosses with the thymoma-free ACI/NMs strain revealed that thymoma development in the BUF/Mna strain was mainly regulated by a dominant susceptibility gene, *Tsr-1*.[2] [*3] Thymomas were assumed to begin to develop in Buffalo rats from 9 months after birth, judging from the weight curve of the thymus.[3] It has, however, been difficult to demonstrate early morphologic changes of the thymuses in BUF/Mna rats, since their thymuses were constitutively too large for detection of initial tumorous foci. Indeed, the thymic involution in this strain was far from being physiologic, leading gradually to genuine thymomas at about 40 weeks of age. On the other hand, ABF1 rats developed overt thymomas much later after an ordinary thymic involution. In this paper, we describe small thymoma nodules detected in these ABF1 rats and discuss the nodular lesions and overt thymomas from the standpoint of tumor progression.

MATERIALS AND METHODS

Inbred BUF/Mna and ACI/NMs rats, and their F1 hybrid rats were used. They were weaned at 4 weeks of age and were given a pellet diet, CMF (Oriental Yeast Co., Tokyo) and tap water freely. Ten to 20 BUF/Mna and ACI/NMs rats of both sexes were killed at appropriate intervals from 3 to 104 weeks of age, and the thymuses or thymomas were weighed and fixed in 10% formalin solution. Twenty, 28, and 36 ABF1 rats were killed at 6, 50, and 104 weeks, respectively, and their thymuses or thymomas were similarly examined. The tissue blocks were processed, and the sections were stained with hematoxylin and eosin (H-E). The thymic ratio was expressed by the thymus weight in mg/body weight in g.[4] Large overt thymomas, which had lost the thymic contour with a rounded

[*3] The locus symbol of *Tbm-1* (thymoma of BUF/Mna rat), provisionally used in the previous papers,[2,4] should be changed to *Tsr-1* (thymoma susceptibility gene of rat), because the symbol of *Tbm* had already been proposed to symbolize "tubular basement membrane antigen" by Matsumoto *et al.*[5]

lower ridge, were diagnosed and subgrouped as large-sized thymomas, following the criteria of Yamada *et al.*,[3] usually weighing more than 2000 mg. Lobular enlargements retaining a thymic contour with a relatively sharp lower ridge were subgrouped as medium-sized thymomas, usually weighing 600–2000 mg. Thymuses which contained small microscopical or macroscopical nodules were subgrouped as small nodules, usually weighing 200–600 mg. These thymomas, including the small nodules, were microscopically confirmed by the criteria of Stewart and Snell,[6] and were classified into 4 types: predominantly lymphocytic, mixed, epithelial, and malignant types, following the classification of Bernatz *et al.*[7]

Results

Thymic Ratio Age-related changes in the thymic ratios of BUF/Mna and ACI/NMs rats are shown in Fig. 1. There was no large sex difference in the ratios (data were combined for Fig. 1). Not only actual thymus weights (Table I), but also the thymic ratios of the BUF/Mna rats were larger than those of the ACI/NMs rats at all the ages examined. In the ACI/NMs rats, the ratio increased until 6 weeks after birth and then decreased rapidly, indicating a physiologic involution. On the other hand, the ratio of the BUF/Mna rats increased sharply until 6 weeks and then decreased, with a great range, much more slowly than that of the ACI/NMs rats. In accordance with the thymoma development, the ratio began to increase again in the BUF/Mna strain from 40 weeks of age. The average thymic ratios of ABF1 rats were intermediate between those of the two parent strains, being in the ranges of 4.5–6.4 at 6 weeks of age (not shown in Fig. 1), 0.3–9.3 at 50 weeks and 0.4–109.2 at 104 weeks (Fig. 1). No sex difference in the ratio was found in these F1 rats either (data were combined).

Structure of Thymus and Thymoma The thymuses of the ACI/NMs rats had a normal structure with a distinct cortex and medulla.

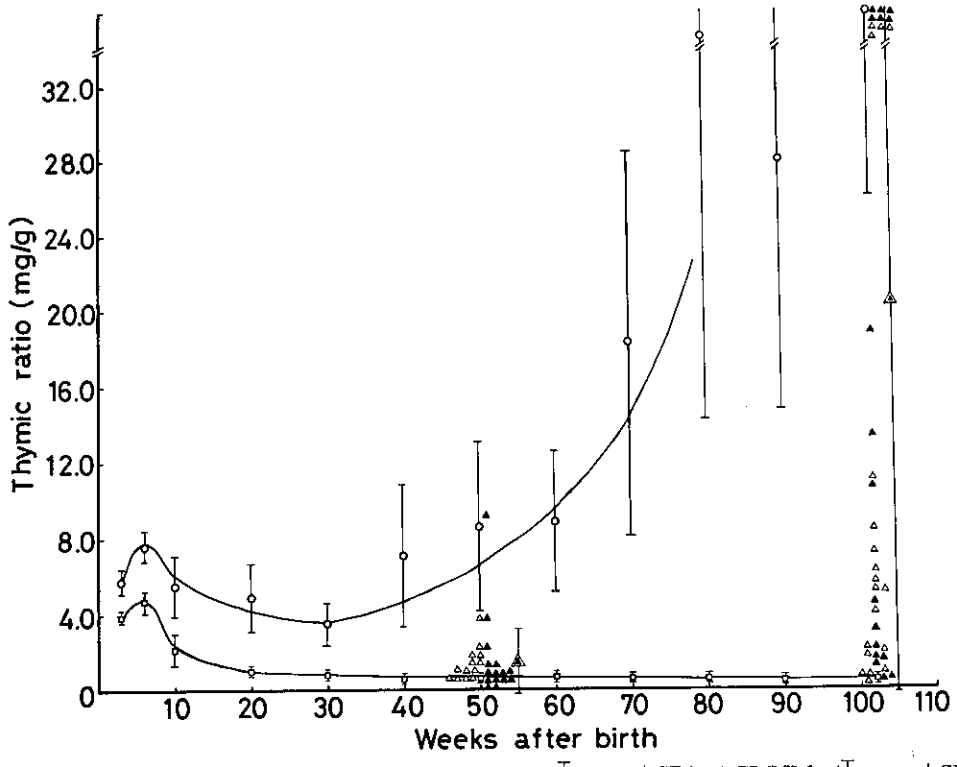

Fig. 1. Changes with age in thymic ratios of BUF/Mna (○ mean±SD), ACI/NMs (□ mean±SD), and ABF1 (△ mean±SD, △ female and ▲ male) rats. There was no large sex difference in the ratios and data for both sexes were combined.

Table I. Thymus Weight and Thymoma Incidence in BUF/Mna, ACI/NMs, and ABF1 Rats

Strain	Weeks after birth	No. of rats	Body weight (g)[a]	Thymus weight (mg)[a]	No. (and %) of rats with			
					Small thymoma nodule[b]	Medium-sized thymoma[b]	Large-sized thymoma[b]	Other tumors[c]
BUF/Mna	3	10(M7, F3)	59±14	351±117	0	0	0	0
	6	17(M8, F9)	136±23	1044±174	0	0	0	0
	10	13(M8, F5)	222±53	1139±189	0	0	0	0
	20	11(M6, F5)	272±80	1517±668	0	0	0	0
	30	17(M8, F9)	298±98	1007±517	0	0	0	0
	40	15(M5, F10)	295±78	2249±1200	1(7)	7(47)	7(47)	0
	50	18(M10, F8)	313±72	2720±1555	0	8(44)	10(56)	0
	60	20(M11, F9)	319±83	2733±1640	0	9(45)	11(55)	0
	70	13(M5, F8)	279±75	5185±4012	0	3(23)	10(77)	0
	80	14(M10, F4)	289±58	11006±7169	0	3(21)	11(79)	4(29)
	90	15(M9, F6)	286±56	10217±6680	0	0	15(100)	6(40)
	104	11(M5, F6)	213±55	10804±4992	0	0	11(100)	6(55)
ABF1	6	20(M10, F10)	117±10	645±76	0	0	0	0
	50	28(M15, F13)	342±108	514±730	15(54)	4(14)	1(4)	0
	104	36(M17, F19)	239±65	4279±6679	7(19)	12(33)	11(31)	19(53)
ACI/NMs	3	20(M11, F9)	39±8	151±29	0	0	0	0
	6	12(M8, F4)	118±20	532±43	0	0	0	0
	10	12(M5, F7)	180±49	360±51	0	0	0	0
	20	10(M5, F5)	218±37	213±52	0	0	0	0
	30	15(M7, F8)	228±50	165±23	0	0	0	0
	40	14(M6, F8)	226±40	142±33	0	0	0	0
	50	13(M5, F8)	233±52	129±18	0	0	0	0
	60	14(M7, F7)	257±60	135±25	0	0	0	0
	70	10(M5, F5)	269±51	134±24	0	0	0	1(10)
	80	13(M5, F8)	256±70	104±16	0	0	0	4(31)
	90	14(M7, F7)	261±46	128±44	0	0	0	8(57)
	104	11(M6, F5)	260±64	86±18	0	0	0	8(73)

a) Values are means±SD.
b) Subgrouped as described in the "Materials and Methods."
c) Contained testicular interstitial cell tumors, pituitary adenomas, mammary fibroadenomas, adrenal pheochromocytomas, and other miscellaneous tumors.

ACI/NMs rats older than 20 weeks had an involuted thymus of age-matched size, leaving narrow cortical areas. On the other hand, BUF-Mna rats between 20 and 40 weeks of age had a thymus with wider cortical areas. All but one of the 106 BUF/Mna rats older than 40 weeks developed medium- and large-sized thymomas and showed increased incidences of large-sized thymoma in advanced age (Table I). The remaining rat had an exceptionally small thymus (526 mg) for a BUF/Mna rat, with 5 microscopic, round nodules. Microscopically, 99 of these 106 thymomas (93%), including the thymus with the small nodules, were of predominantly lymphocytic type, showing cortex-like appearance with a starry-sky pattern. No definite medullae were observed in these thymomas, but small round areas similar to the "foci of medullary differentiation" described in human thymomas,[8] were frequently found. Others were 4 of mixed type, 2 of epithelial type, and 1 of malignant type, the last of which showed a carcinosarcomatous pattern; this rat had metastases to the mediastinum and into the intercostal muscles.

Out of 28 ABF1 rats killed at 50 weeks of age, only 5 rats (18%) developed overt

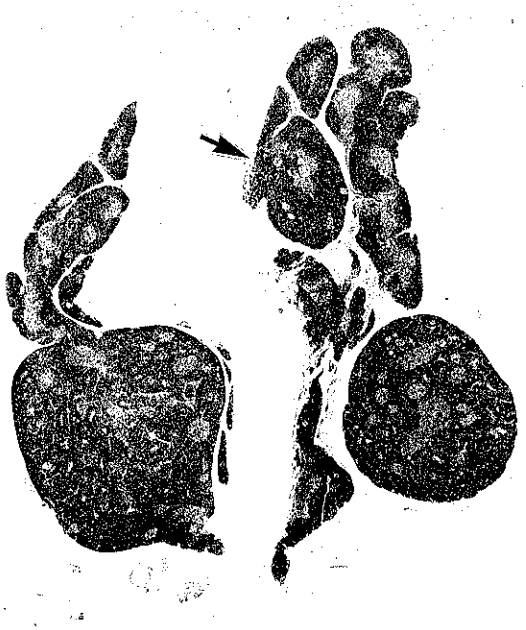

Fig. 2. Three round thymoma nodules of a male ABF1 rat 50 weeks old (thymus weight: 314 mg; thymic ratio: 0.7). The nodule (arrow) of the upper part appears to arise eccentrically from the involuted thymic lobule, while the larger nodules of lower parts compress the remaining lobules. H-E. ×7.6.

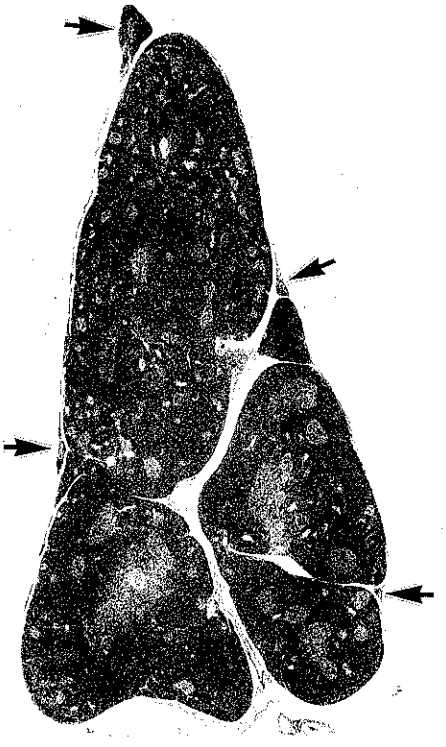

Fig. 3. A whole left lobe of a medium-sized thymoma in a male ABF1 rat 50 weeks old (weight, 1835 mg; ratio, 3.8). Fused nodules, retaining a thymic contour, and severely involuted thymic lobules, which are flattened layers and sometimes triangular in shape (arrows), can be seen. H-E. ×8.0.

thymomas, 4 medium-sized (Fig. 3) and one large-sized (Table I). Fifteen (54%) had small nodules in their involuted thymuses (Fig. 2), ranging from 1 to 13 in number. The nodules were round in shape; the relatively large ones, about 5 mm or more in diameter, were macroscopically discernible, but the smaller ones could only be detected by microscopic examinations. They consisted of cortex-like tissue packed with a large number of normal-looking small lymphocytes in a network of polygonal-shaped epithelial cells. The nuclei of epithelial cells often looked larger than those in the adjacent involuted cortex (Fig. 4). Severely involuted thymus lobules were frequently observed around the nodules or lobular masses of medium-sized thymomas (Figs. 2 and 3). All of these 20 thymomas, including the thymuses with the small nodules, were of predominantly lymphocytic type.

When killed at 104 weeks of age, 23 of 36 ABF1 rats (64%) had developed medium to large thymomas and 7 other rats (19%) had small thymoma nodules (Table I). Twenty-six of these 30 thymomas were diagnosed as predominantly lymphocytic, 2 as mixed, 1 as epithelial (Fig. 5), and 1 as malignant type with the histologic features of squamous cell carcinoma, metastasizing to the intercostal muscles (Fig. 6). No spindle cell type[7] was found in the thymomas.

Testicular interstitial cell tumors, pituitary adenomas, mammary fibroadenomas, adrenal pheochromocytomas, and other miscellaneous tumors were found in some rats of these strains at over 70 weeks of age (Table I).

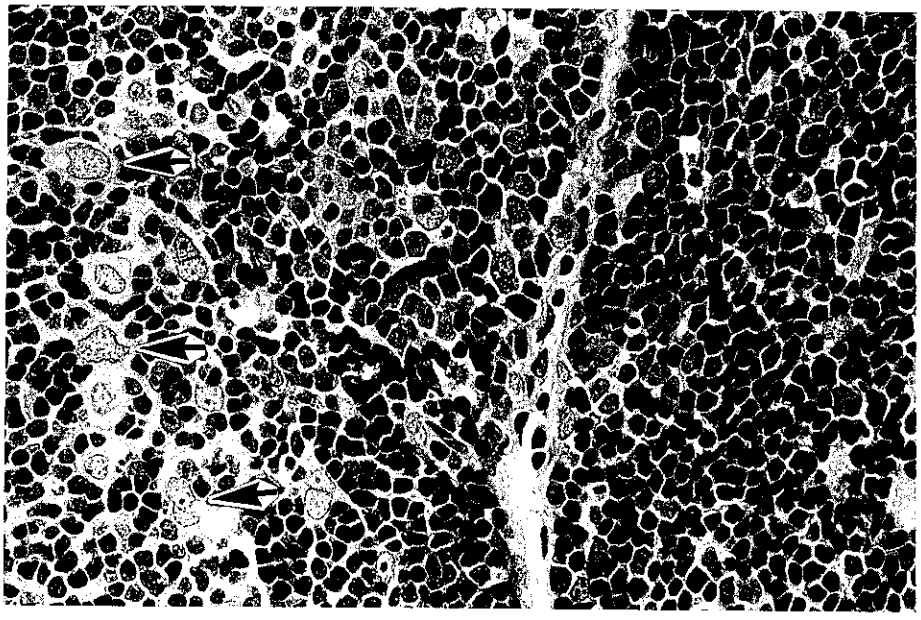

Fig. 4. Thymoma nodule of a male ABF1 rat of 50 weeks old (weight, 455 mg; ratio, 0.9). Nodule (left two-thirds) is composed of a great number of small lymphocytes and fewer epithelial cells. Some epithelial cells (arrows) in the nodule have larger nuclei than those in involuted thymic tissues. H-E. ×680.

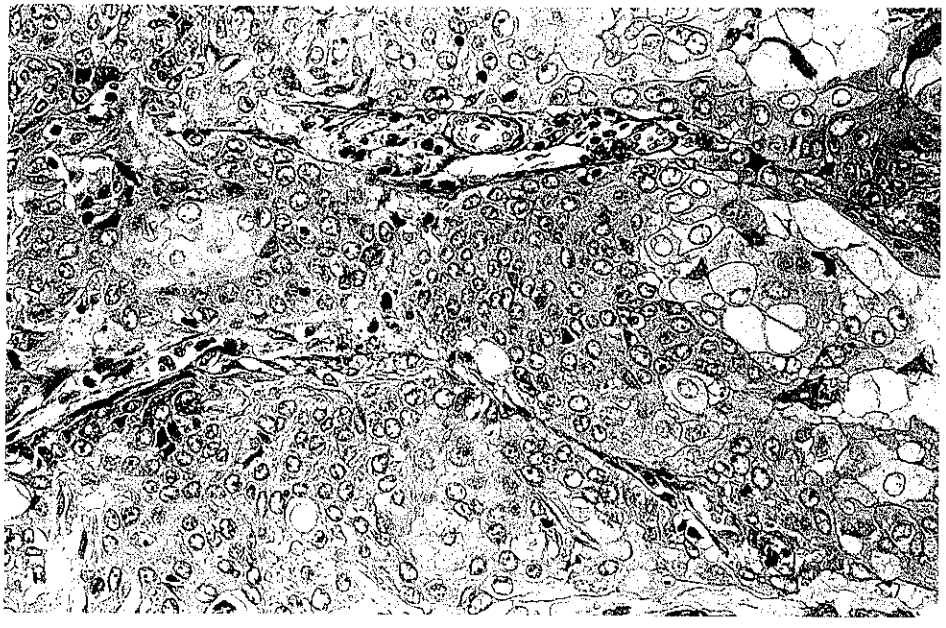

Fig. 5. An area of a medium-sized thymoma in a female ABF1 rat 104 weeks old (weight, 1270 mg; ratio, 8.4). The thymoma consists exclusively of polygonal epithelial cells. H-E. ×350.

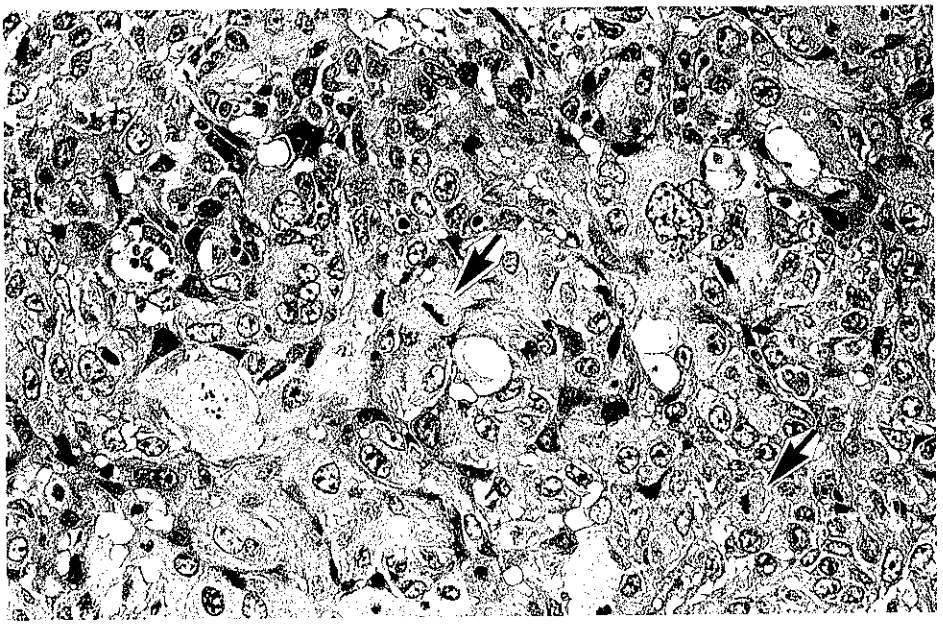

Fig. 6. An infiltrated area in the intercostal muscles from a medium-sized thymic tumor in a male ABF1 rat 104 weeks old (weight, 1260 mg; ratio, 4.2). Tumor cells vary greatly in size and have large atypical nuclei, with a few mitotic figures (arrows). H-E. ×350.

Discussion

The neoplastic nature of human thymoma has been widely acknowledged, and it sometimes develops as thymoma nodules in the initial stage.[7] On the other hand, no such evidence has been reported concerning rat thymomagenesis,[3, 9–12] leaving the assumption that rat thymoma might be hyperplastic in nature. The results of the present study, however, showed that thymomas of ABF1 rats also began with small nodules. These nodules were of multiple origin and were assumed to develop under the influence of a susceptibility gene, *Tsr-1*, originating from the BUF/Mna father. *Tsr-1* was apparently dominant in its thymoma-producing effect, at least in the combination of the BUF/Mna and ACI/NMs strains.[2] Although the physiologic function of its normal allele is unknown, the mutant allele, *Tsr-1* may possibly enhance the proliferative capacity of the epithelial cells.[4] Therefore, it is conceivable that the thymomas of BUF/Mna rat in fact occurred multicentrically as small thymoma nodules, but that the detection of the nodules was difficult due to the constitutively large thymuses. The use of the ABF1 rats seemed to be of advantage in order to see the initial stage of rat thymomagenesis, because in the heterozygous state, the effect of *Tsr-1* might be just above the threshold, making the detection of nodules possible. A rise in the thymic ratios from the age of 50 weeks in the ABF1 and 40 weeks in the BUF/Mna rats can be interpreted as showing that the small nodules develop into medium to large thymomas as a result of growth and fusion. Furthermore, a few mixed, epithelial, and malignant types of thymomas developed in these rats. These findings may suggest that the mechanism of "tumor progression"[13–15] is operating in some cases of rat thymomas, from predominantly lymphocytic to mixed, epithelial, and malignant types, as assumed in human thymoma development.[16–18]

It was previously shown that the epithelial cells from thymomas of old BUF/Mna rats cultured for 1 week were much larger than those cultured from normal thymuses and

that they had larger nuclei or were binuclear.[19] The latter point was confirmed in the present *in vivo* study by examining the thymoma and adjacent non-thymoma tissues histologically. The epithelial cells of both small thymoma nodules and medium to large thymomas often had larger or atypical nuclei compared with those of the adjacent involuted thymuses. Furthermore, thymomas did develop from the fetal thymic rudiments of BUF/Mna rats under the kidney capsule of ACI/NMs-*rnu/rnu* rats, apart from the BUF/Mna environment. Reactive hyperplasia is no longer a likely explanation for these particular thymomas. These thymomas, as was expected, consisted of epithelial cells of donor type and lymphocytes of host type (O. Taguchi *el al.*, unpublished data). These observations may also support our concept that the thymoma of the BUF/Mna rat is not simply hyperplastic, but potentially neoplastic in nature. Although further efforts are needed to get definitive evidence for the neoplastic nature of rat thymomas, no appropriate method is at present available in rats to examine the clonality of thymic epithelial cell nodules.

The present study also showed that the small nodules, initial lesions of rat thymomagenesis, were always of predominantly lymphocytic type. This coincides with the fact that cultured epithelial cells from BUF/Mna thymomas produce a biologically active substance which could endow immature lymphoid cells with non-specifically proliferating, concanavalin A-responding and rosette-forming capacities.[20]

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