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An Intrinsic Thymic Epithelial Abnormality Is Responsible for the Spontaneous Development of Predominantly Lymphocytic Thymomas in BUF/Mna Rats

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The nature of tumorigenesis of predominantly lymphocytic thymoma was examined using an animal model. Rats of the inbred BUF/Mna strain were found spontaneously to develop predominantly lymphocytic thymomas, histologically indistinguishable from their human counterparts, at an incidence of virtually 100%. Thymic rudiments of BUF/Mna rats grafted 17 months previously under the renal capsule of young athymic ACI/NMs-*rnu/rnu* rats also gave rise to similar lesions. The lymphocytes in the thymomas expressed T-cell antigens (rat Lyt-1 and Lyt-2.3), as in the normal case, and ACI rat specific antigen. When BUF/Mna rats of thymoma age were irradiated with a lethal dose of 12 Gy and then received a single injection of bone marrow cells (8×10^7) from BALB/c-*nu/nu* mice, thymomas were re-formed three weeks later (in 2 of 5 rats) with the replacement lymphocytes expressing mouse Thy-1.2 antigen. These results indicate that an intrinsic thymic epithelial abnormality is responsible for the development of predominantly lymphocytic thymomas in BUF/Mna rats.

Key words: Thymomagenesis — Predominantly lymphocytic thymoma — BUF/Mna rat — Bone marrow transplantation — Athymic nude rat

Thymomas developing in humans are classified histologically into polygonal and spindle epithelial cell types, the former being further divided into predominantly lymphocytic, mixed lymphoepithelial and predominantly epithelial cell categories.[1] The predominantly lymphocytic type constitutes about one-fourth of human thymomas.[1] Histologically very similar thymomas have been described to develop spontaneously in a colony and a few inbred strains of rats,[2-6] and we have established a line of the BUF/Mna strain which is particularly prone to predominantly lymphocytic type thymoma development.[4] Thus a virtually 100% incidence can be obtained, although the question of which cells, thymic lymphocytes from bone marrow, or the thymic environment including epithelial cells, or both, are actively involved in the tumorigenesis of this type of lesion remains unclear. Using our BUF/Mna rats, we aimed to clarify this critical point regarding thymomagenesis. Development of thymomas from thymic rudiments grafted under the renal capsule of allogeneic athymic nude (*rnu/rnu*) rats, and successful re-formation of thymoma by xenogeneic bone marrow transplantation[7,8] demonstrated that thymic epithelial abnormalities themselves are associated with predominantly lymphocytic thymoma.

MATERIALS AND METHODS

Animals The thymoma-prone inbred line of BUF/Mna rats[4] and BUF/Mna-*rnu/rnu* rats[9] established in the Aichi Cancer Center Research Institute, thymoma-free control inbred lines of ACI/NMs and F344/DuCrj rats purchased from Japan Charles River Inc., Hino, ACI-*rnu/rnu* rats purchased from CLEA Japan Inc., Kanagawa and BALB/c-*nu/nu* mice purchased from Japan Charles River Inc. were used in this experiment. The animals were all housed in specific pathogen-free rooms.

Thymic grafting Thymus lobes harvested from 15-day embryos of BUF/Mna, ACI/NMs or F344/DuCrj rats were grafted under the renal capsule of 4-week-old BUF/Mna-*rnu/rnu* and ACI/NMs-*rnu/rnu* rats. The thymus-grafted nude rats were killed at 18 months of age.

Bone marrow transplantation Production of xenogeneic mouse-to-rat bone marrow chimeras was achieved using modifications of the techniques developed by Pollard *et al.*[7] Five 12-month-old BUF/Mna rats were irradiated with a lethal dose of X-ray (12 Gy, Hitachi MBR-1520R; Hitachi, Tokyo) and 1 day later injected intravenously with 8×10^7 bone marrow cells from 2 month-old BALB/c-*nu/nu* mice. The rats were killed 3 weeks after the injection.

Serological analysis The surface antigens of lymphocytes in normal thymi of 2-month-old BUF/Mna and ACI/NMs rats, thymomas of BUF/Mna rats, thymomas

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which developed under the renal capsule of allogeneic nude rats, and thymomas re-formed by xenogeneic bone-marrow transplantation were analyzed. Polyclonal antiserum against ACI/NMs rat cell specific antigen was prepared by 4 immunizations of BUF/Mna rats at 4-week intervals with spleen cells (2×10^7) prepared from ACI/NMs rats. Analysis of cell surface antigens was performed with a fluorescence-activated cell sorter (FACS) (FACS IV, Becton Dickinson & Co., Mountain View, CA) as described previously[10] using polyclonal antiserum to ACI/NMs rat cell specific antigen, monoclonal antibodies to rat Lyt-1 (mouse IgG2a, R1-3B3) and rat Lyt-2.3 (mouse IgG2a, R1-10B5) (gifts from Dr. K. Kikuchi, Sapporo Medical College), and fluorescein isothiocyanate (FITC)-labeled monoclonal antibodies to rat leukocyte common antigen (Serotec, Oxford, England), rat IgG (Cappel, Organon Teknika, West Chester, PA), mouse Thy-1.2 (Becton Dickinson), and mouse IgG (Cappel, Organon Teknika). As secondary reagents for polyclonal and monoclonal antisera, FITC-labeled anti-rat IgG and anti-mouse IgG were used, respectively.

Immunohistochemistry Two thymomas harvested from xenogeneic bone marrow transplanted BUF/Mna rats were embedded in O.T.C. Compound (Tissue Tek II, Naperville, IL) and immediately frozen in liquid nitrogen. Control thymus tissues were also obtained from 2-month-old BALB/c mice and BUF/Mna rats. Sections were incubated with anti-rat species specific antisera,[11] washed, then incubated with rhodamine-labeled anti-rabbit IgG (Cappel, Organon Teknika) and FITC-labeled anti-Thy 1.2 monoclonal antibody in a double-staining method.

Histology Tissues were fixed in Bouin's fixative, embedded in paraffin, sectioned, and stained with hematoxylin and eosin for histological examination.

RESULTS

Spontaneous development of thymoma As summarized in Table I, spontaneous development of thymomas was found in 100% of BUF/Mna rats aged 12 months and above, whereas no thymic abnormalities were found in ACI/NMs or F344/DuCrj animals. Histologically, the

Table I. Thymoma Incidence

Rats	No. of rats with thymoma	
	at 12 months	at 18 months
BUF/Mna	7/7	5/5
ACI/NMs	0/5	0/20
F344	0/5	0/15

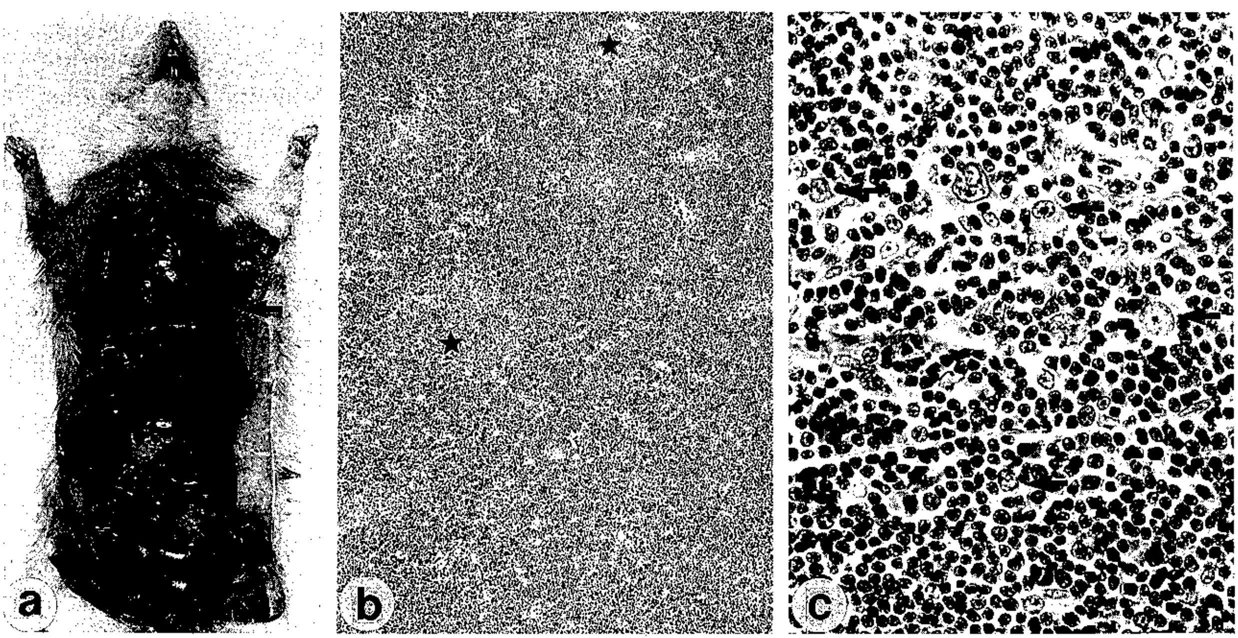


Fig. 1. A thymoma found in an 18-month-old male BUF/Mna rat. (a), macroscopic appearance (arrow). (b), histological features of the predominantly lymphocytic type lesion with small round and low-density areas (stars). ×75. (c), higher power image of (b). Arrows indicate epithelial cells with larger nuclei. ×560.

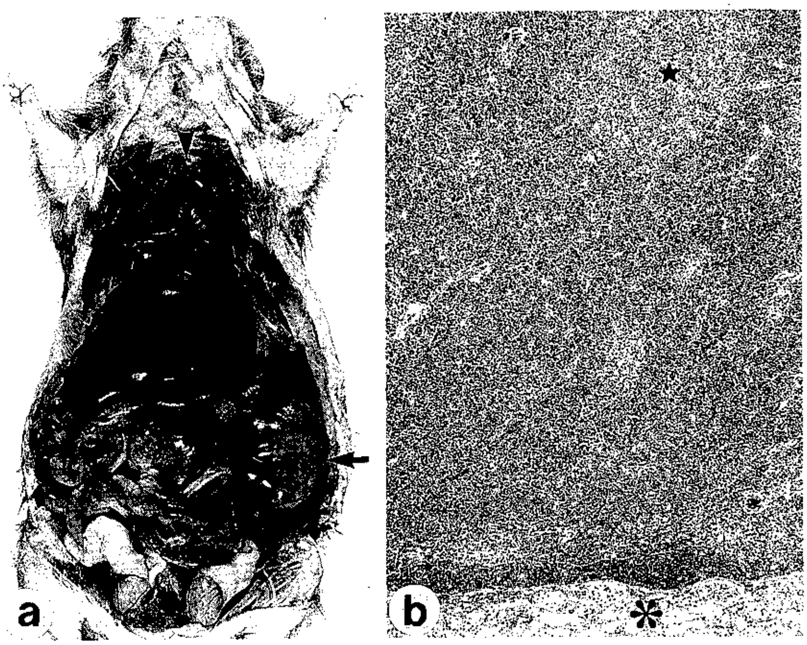


Fig. 2. A thymoma developing from an embryonic BUF/Mna thymus that had been grafted under the renal subcapsule of a male ACI/NMs-*rnu/rnu* rat 17 months previously. (a), macroscopic appearance of the lesion (arrow) and athymia (arrowhead). (b), histological features showing the small round and low-density areas (star) of a predominantly lymphocytic type with an adjacent area of kidney (asterisk). ×75.

thymomas were all of predominantly lymphocytic type as illustrated in Fig. 1, showing a cortex-like appearance with a "starry-sky" pattern (Fig. 1b and c). No definite medullae were observed in these thymomas, but small round and low-density areas similar to the foci of "medullary differentiation" described in human thymomas[1] were frequently observed (Fig. 1b).

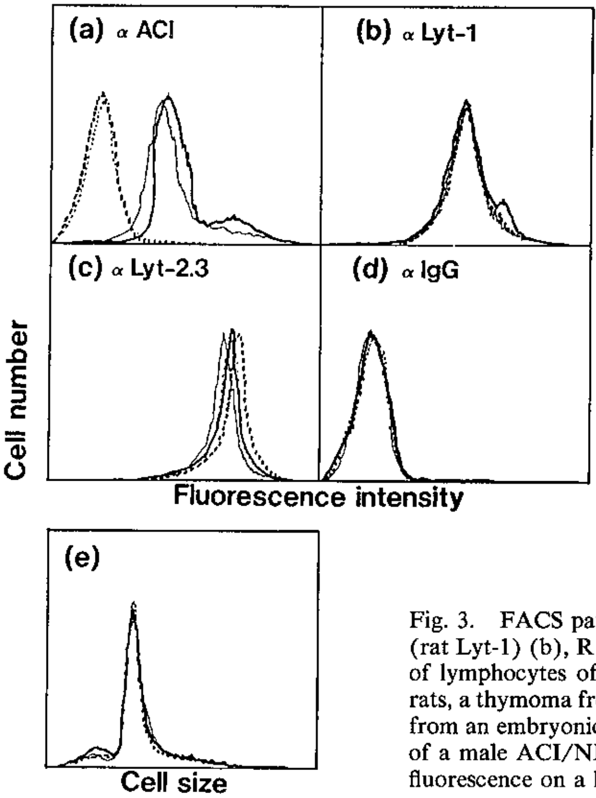
Development of thymoma from thymic allografts To determine whether stem cells derived from the bone marrow (T cells, dendritic cells and macrophages) or the thymic epithelial cells of BUF/Mna rats were responsible for the thymomagenesis, thymus grafting into allogeneic nude rats was carried out. As shown in Table II, predominantly lymphocytic thymomas developed from BUF/Mna thymic rudiments that had been grafted under the renal capsule of both ACI/NMs (Fig. 2a and b) and BUF/Mna nude rats. On the other hand, only atrophic thymi were found when thymic rudiments from either ACI/NMs or F344/DuCrj rats had been grafted under the renal capsule of athymic ACI-*rnu/rnu* or BUF/Mna-*rnu/rnu* rats.

Table II. Development of Thymoma from Thymic Allografts in Athymic Nude Rats

Thymic donor	Host	Fate of the grafted thymi (No. thymomas/No. hosts)
BUF/Mna	ACI/NMs-*rnu/rnu*	Thymoma (6/6)
ACI/NMs		Atrophy (0/6)
F344/DuCrj		Atrophy (0/4)
BUF/Mna	BUF/Mna-*rnu/rnu*	Thymoma (5/5)
ACI/NMs		Atrophy (0/5)
F344/DuCrj		Atrophy (0/5)

Both thymus lobes harvested from 15-day-old embryonic BUF/Mna, ACI/NMs or F344/DuCrj rats were grafted under the renal capsule of 5-week-old athymic nude rats of the ACI/NMs or BUF/DuCrj strain. These rats were then killed at 18 months of age.

To determine the origin of lymphocytes in the thymomas developing from thymic allografts (BUF/Mma thymus → ACI-*rnu/rnu* kidney), their surface antigens were analyzed by FACS. As shown in Fig. 3a, the lymphocytes in the thymomas possessed ACI/NMs rat antigens and expressed both rat Lyt-1 and Lyt-2.3 antigens at similar levels to those in original thymomas *in situ* and also normal thymocytes of young ACI/NMs and BUF/Mna rats (Fig. 3b and c). No massive infiltration of B cells was observed (Fig. 3d). In addition, no difference in cell size was noted among these lymphocytes (Fig. 3e). These results clearly indicate that the thymic epithelial cells of BUF/Mna rats were involved in the thymoma-



genesis of predominantly lymphocytic-type lesions. Furthermore, their nature was confirmed by the radiation chimera experiments.

Re-formation of thymoma by xenogeneic bone marrow transplantation Re-formation of predominantly lymphocytic thymoma was observed in 2 of 5 lethally irradiated BUF/Mna rats after transplantation of bone marrow cells from xenogeneic BALB/c nude mice (BALB/c → BUF/Mna) (Fig. 4a). The other animals died about 10 days after the treatment without thymic re-formation. Histological features of the reformed thymomas were similar to those of the spontaneously developing thymomas. Treatment of frozen sections through these thymomas by the double-staining immunofluorescence technique with antisera against rat species specific antigen and mouse T-cell specific antigen (Thy-1.2) revealed the reticular meshwork structure to possess rat species

Fig. 3. FACS pattern of the expression of ACI/NMs rat specific antigen (a), R1-3B3 (rat Lyt-1) (b), R1-10B5 (rat Lyt-2.3) (c) and rat IgG (d), and of relative cell sizes (e) of lymphocytes of thymi from 2-month-old BUF/Mna (·······) and ACI/NMs (——) rats, a thymoma from an 18-month-old BUF/Mna rat (----) and a thymoma developing from an embryonic BUF/Mna rat thymus that had been grafted under the renal capsule of a male ACI/NMs nude rat 17 months previously (——). a–d: the x axis is relative fluorescence on a log scale; the y axis is cell number.

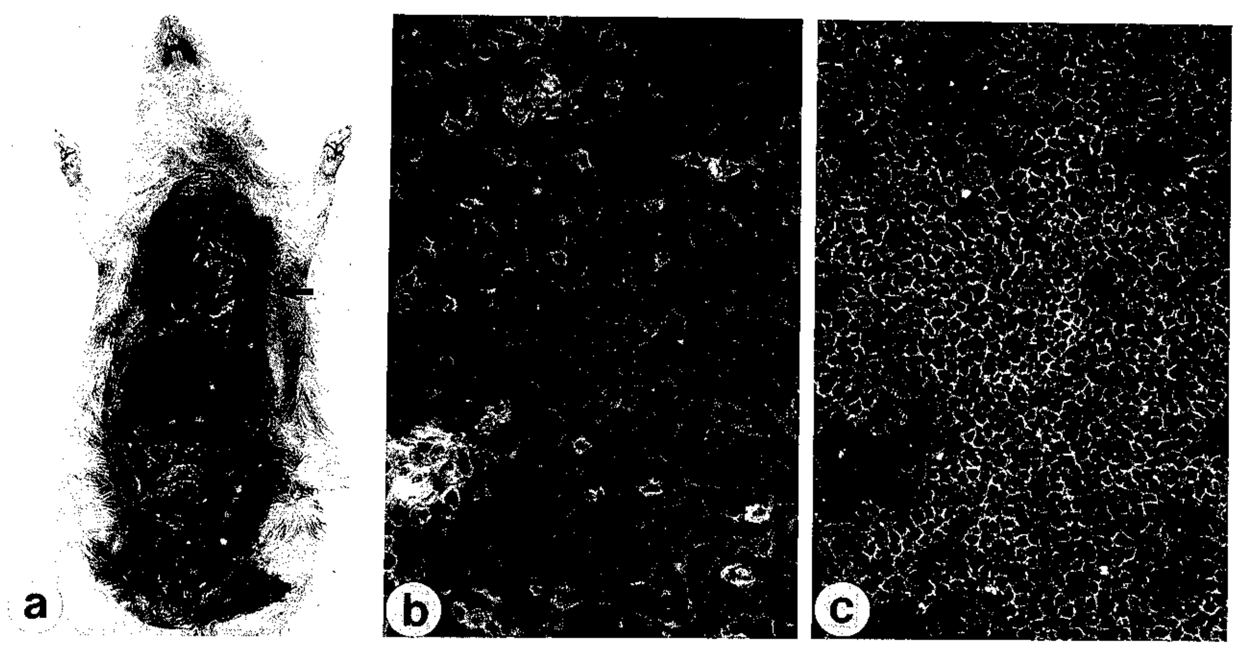


Fig. 4. Macroscopic appearance of a thymoma in a 13-month-old BUF/Mna rat reformed by xenogeneic bone marrow transplantation (BALB/c→BUF/Mna) (a). A frozen section of the thymoma shown in (a) stained simultaneously with anti-rat species-specific antigen antiserum (rhodamine) (b) and anti-Thy-1.2 antiserum (FITC) (c). ×550.

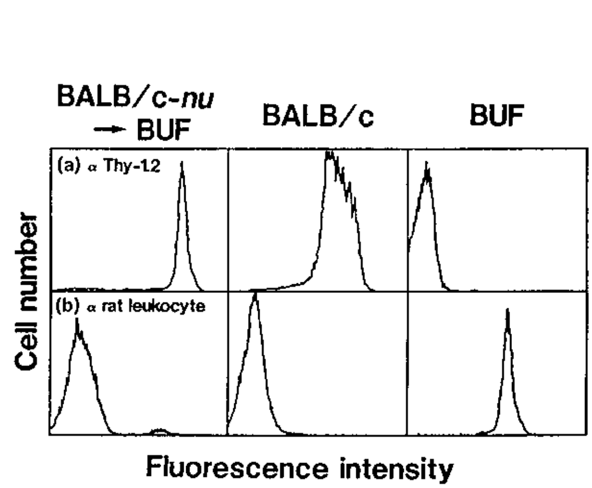


Fig. 5. FACS pattern of the expression of Thy-1.2 (a) and rat leukocyte antigens (b) on lymphocytes of the thymi of a 2-month-old BALB/c mouse and a BUF/Mna rat, and of a re-formed thymoma in a 13-month-old BUF/Mna rat after xenogeneic bone marrow transplantation (BALB/c-*nu/nu*→BUF/Mna). The *x* axis is relative fluorescence on a log scale; the *y* axis is cell number.

specific antigens (Fig. 4b) while the thymocytes were Thy-1.2 antigen-positive (Fig. 4c). Furthermore, flow cytometric analysis confirmed the immunohistochemical result, showing that about ninety-five percent of lymphocytes in the reformed chimeric thymomas were Thy-1.2-positive cells of donor origin (Fig. 5a). No graft-versus-host like reaction was observed in the rats.

DISCUSSION

We earlier established the BUF/Mna strain as a thymoma-prone inbred line of rats.[4] In the present study, all BUF/Mna rats older than 12 months developed a thymoma histologically classified as of predominantly lymphocytic type. Several stimuli from extra-thymic tissues, such as hormones from the pituitary gland, are considered crucial for thymomagenesis in BUF/Mna rats.[12] However, grafts of embryonic BUF/Mna rat thymi gave rise to thymomas in an allogeneic nude host environment, whereas, in clear contrast, no lesions developed from ACI/NMs or F344/DuCrj rat thymus grafts in BUF/Mna nude rat hosts. These thymic graft experiments unequivocally demonstrated that it is the thymus *per se* which is indispensable for thymomagenesis in BUF/Mna rat.

Analysis by FACS of the surface antigens of lymphocytes in the thymomas that developed in an allogeneic host environment revealed that host lymphocytes migrated into thymomas and expressed T-cell antigens as in normal thymus thymocytes. Recently, we showed that the host-T-cell function of BALB/c nude mice can be re-established by implantation of xenogeneic thymic rudiments from embryonic F344 rats.[11] The grafted rat thymi developed well and formed cortex and medulla zones, with the lymphocytes, dendritic cells and macrophages being eventually entirely replaced with host types (unpublished data). From these observations, we believe that not only the lymphocytes but also the dendritic cells and macrophages in the thymomas developing in allogeneic hosts were of host origin. These results strongly suggest that the thymic epithelial cells and not the stem cells of bone marrow are responsible for thymomagenesis in BUF/Mna rats. Furthermore, the radiation chimera experiment confirmed the existence of a thymic epithelial abnormality in BUF/Mna rats. Analysis by FACS of the re-formed chimeric thymomas produced by xenogeneic bone marrow transplantation (BALB/c nude→BUF/Mna) revealed that while the lymphocytes in the chimeric thymomas were of donor mouse type, the indispensable thymic epithelial cells were from the BUF/Mna hosts, the epithelial cells forming thymomas thus possessing the ability to attract T precursor cells.

It was earlier demonstrated by crosses with the thymoma-free ACI/NMs strain that thymomagenesis in the BUF/Mna strain is mainly regulated by a dominant susceptibility gene, *Tsr-1* (formerly *Tbm-1*).[13] The present results imply that expression of this *Tsr-1* gene in thymic epithelial cells warrants further investigation in regard to its role in genesis of this important type of thymoma.

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