Growth and Metastasis of Two Canine Mast Cell Tumors in SCID Mice

Kohji MARUO, Tsuyoshi SUGIMOTO, Kaoru SUZUKI, Kinji SHIROTA¹⁾, Yoshihisa YAMANE, and Tatsuji NOMURA²⁾

Department of Veterinary Surgery, Faculty of Agriculture, Tokyo University of Agriculture and Technology, 3-5-8 Saiwai, Fuchu, Tokyo 183,

¹⁾Department of Veterinary Pathology, Azabu University School of Veterinary Medicine, 1-17-71 Fuchinobe, Sagami-hara, Kanagawa 229, and ²⁾Central Institute for Experimental Animals, 1430 Nogawa, Miyamae-ku, Kawasaki, Kanagawa 213, Japan

(Received 6 February 1995/Accepted 18 January 1996)

ABSTRACT. Intermediately differentiated mast cell tumors in two dogs were subcutaneously xenotransplanted into severe combined immunodeficiency (SCID) mice. Both tumors primarily grew and were serially transplantable in SCID mice. The histological features of the xenografts were similar to those of original tumors in dogs. Both of these subcutaneous tumors were judged as connective tissue mast cells by toluidine blue stain. One of the two xenografts metastasized to the tracheobronchial lymph nodes, omentum, mesentery, subpleural region and retroperitoneum of the SCID mouse. These canine mast cell tumor xenografts in SCID mice may be valuable tools for investigating the growth and metastatic behaviors of the tumor. — **KEY WORDS:** canine mast cell tumor, mouse (SCID), xenotransplantation.

J. Vet. Med. Sci. 58(6): 567–569, 1996

Mast cell tumors are very common in dogs. The incidence of mast cell tumor in the dog has been reported to be 7 to 21% of all skin tumors and 11 to 27% of all malignant skin tumors [12]. The canine mast cell tumors have a variety of histologic patterns and are classified into three histologic gradings based on the degree of differentiation by Bostock [1] and Patnaik *et al.* [9]. The histologic grading is known to be helpful in predicting biologic behavior. Recurrence after the resection of primary tumor is very common in cases with high-grade canine mast cell tumors [1]. Therefore, it is necessary to develop new therapies against these mast cell tumors. In addition, degranulation of the tumor cells resulting in release of the chemical mediators such as histamine and heparin is well known to cause paraneoplastic syndromes including gastrointestinal ulcerations and coagulopathies in the host [12]. Mechanism of degranulation of mast cells has been investigated in the fields of oncology and immunology [2, 4].

On the other hand, tumor xenografts have been widely used as tools for the investigation of tumor cells [3, 10]. As recipients of xenografts, severe combined immunodeficiency (SCID) mice deficient in both T and B cell functions have

been shown to be more useful than athymic nude mice deficient in T cell function [7, 13]. In the present study, we transplanted canine mast cell tumors into SCID mice and established two xenograft lines.

Female C.B-17 SCID mice (four to nine weeks old) were obtained from the Central Institute for Experimental Animals in Kawasaki, Japan. Mice were maintained in specific pathogen-free conditions as previously described [11]. The profiles of the two canine mast cell tumors xenotransplanted were summarized in Table 1. Both cases showed recurrence 2–3 months after surgery and subsequently resulted in poor prognosis despite further operation. Other postoperative treatments such as irradiation and chemotherapy were not sufficiently performed in both cases. The skin tumors were resected from two dogs at the first surgery. The tumors were diagnosed as mast cell tumors of intermediately differentiated (histologic grade II) classified by Patnaik *et al.* [9] (Fig. 1). They were cut into 3 × 3 × 3 mm in size and were subcutaneously transplanted into the right flank of two or three SCID mice under sterile conditions. The sex of the recipient mice was matched to that of the donor dogs. The xenografts which formed a nodule of about 3 cm in diameter were transferred to another SCID mouse in the

Table 1. Profiles of two dogs with mast cell tumor and their xenografts in SCID mice

Clinical profile								Transplantation data					
No.	Breed	Sex	Age (Years old)	Primary site	Stage ^{a)}	Histologic grade ^{b)}	Prognosis	Xenograft	Transfer interval (weeks)	Passage No.	Rate ^{d)}	Meta. ^{c)}	Frozen preserva- tion ^{f)}
1	Kishu	F	7	Skin, Flank	IIa	Grade II	Recurrence, dead (5mo) ^{c)}	CMC-1	7–9	9	19/19	–	+
2	Shiba	F	11	Skin, Forelimb	IIa	Grade II	Recurrence, euthanasia(9mo) ^{c)}	CMC-2	12–14	6	16/16	+	+

a) Determined by WHO TNM classification of tumors in domestic animals [8].

b) According to the classification by Patnaik *et al.* [9].

c) Months after first operation.

d) No. of mice with tumor progression/No. of mice inoculated tumor.

e) Metastasis in SCID mice inoculated tumor.

f) Whether tumor fragments frozen in liquid nitrogen and thawed grow in SCID mice or not.

Fig. 1. Mast cell tumor in the dog No. 1 (A) and its xenograft primarily grown in a SCID mouse (B). Mast cell tumor in the dog No. 2 (C) and its xenograft at fourth passage (D). Toluidine blue stain (pH=4.1), $\times 460$.

Fig. 2. Metastatic lesion of canine mast cell tumor xenograft (CMC-2) around the esophagus and trachea of a SCID mouse approximately six months after the third passage, HE stain, $\times 41$.

same way. If tumor growth was not confirmed by 4 months after transplantation, the transplantation was judged to be unsuccessful. As an index of the growth speed of xenografts, the transfer intervals were recorded. All tumor-bearing SCID mice were autopsied and carefully examined for metastasis. Original tumors, subcutaneous xenografts and metastatic lesions in the SCID mice were fixed in 4% paraformaldehyde and embedded in paraffin. Four- μ m tissue sections were stained with hematoxylin and eosin, and toluidine blue (pH=4.1).

Mast cell tumors primarily transplanted in SCID mice showed an obvious growth and could be passaged to further generations. Serial transplantation in SCID mice and frozen preservation in liquid nitrogen were successful in both xenografts. The established xenografts originating from case

No. 1 and No. 2 were named as CMC-1 and CMC-2, respectively (Table 1). The transfer intervals of these xenografts became to be gradually shorter and then to be constant after several passages. The successful transplantation rates of CMC-1 and CMC-2 in SCID mice were 100% at ninth and sixth generations, respectively. The two xenografts consisted of cells with variable size and having larger nuclei and few small cytoplasmic granules, showing characteristics similar to those of their original tumors (Fig. 1). But the mitoses were more frequent in the xenografts than that in their original tumors. As both cytoplasmic granules of original tumors and their subcutaneous xenografts were stained purple with toluidine blue (Fig. 1), these tumors were determined as connective tissue mast cells [6]. Metastasis occurred in SCID mice transplanted with CMC-2 six months after the third passage, and the metastatic lesion was observed in the tracheo-bronchial lymph nodes (Fig. 2), omentum, mesentery, subpleural region and retroperitoneum. However, metastasis was not seen in SCID mice with CMC-1 even through the nine transfers. None of SCID mice autopsied had gastrointestinal ulcers and coagulopathies.

Establishment of canine mast cell tumor xenograft lines was shown in a report using athymic nude mice [5]. Successful primary transplantation rates of canine mast cell tumors in nude mice [5] and SCID mice [11] were shown to be 41.7% (5/12) and 60% (3/5), respectively, although their histological grading was unclear. It has been reported that the tumors transplanted in nude mice grow only in the subcutaneous region [5]. In addition, the tumor-bearing nude mice did not have any metastatic lesions [5]. On the other hand, the tumor xenograft transplanted in SCID mice metastasized in our present study. This evidence may indicate an important role of B cells for metastasis. Further studies are necessary to investigate mechanism of the

enhanced metastatic behavior in SCID mice.

We established two canine mast cell tumor xenografts using SCID mice in the present study. These xenografts may greatly contribute to the research on the growth and metastatic behaviors, and therapies of canine mast cell tumors.

REFERENCES

1. Bostock, D. E. 1973. *J. Small Anim. Pract.* 14: 27-40.
2. Demora, F., Garcia, G., Ferrer, L., and Arboix, M. 1993. *Vet. Immunol. Immunopathol.* 39: 421-429.
3. Goldin, A., Venditti, J. M., Macdonald, J. S., Muggia, F. M., Henney, J. E., and Devita, V. T. 1981. *Eur. J. Cancer* 17: 129-142.
4. Inase, N., Schreck, R. E., and Lazarus, S. C. 1993. *Am. J. Physiol.* 264: 387-390.
5. Lazarus, S. C., DeVinney, R., McCabe, L. J., Finkbeiner, W. E., Elias, D. J., and Gold, W.M. 1986. *Am. J. Physiol.* 251 (Cell Physiol. 20): C935-C944.
6. Miller, H. R. P. and Walshaw, R. 1972. *Am. J. Pathol.* 69: 195-208.
7. Nomura, T., Takahama, Y., Hongyo, T., Takatera, H., Inohara, H., Fukushima, H., Ono, S., and Hamaoka, T. 1991. *Jpn. J. Cancer Res.* 82: 701-709.
8. Owen, L. N. 1989. pp. 448-489. In: *Clinical Veterinary Oncology* (Withrow, S. J. and MacEwen, E. G. eds.), J. B. Lippincott Company, Philadelphia.
9. Patnaik, A. K., Ehler, W. N., and MacEwen, E. G. 1984. *Vet. Pathol.* 21: 469-474.
10. Sharkey, F. E. and Fogh, J. 1984. *Cancer Met. Rev.* 3: 341-360.
11. Sugimoto, T., Maruo, K., Imaeda, Y., Suzuki, K., Shiota, K., Ejima, H., Endo, S., and Nomura, T. 1994. *J. Vet. Med. Sci.* 56: 1087-1091.
12. Tams, T. R. and Macy, D. W. 1981. *The Compendium Continuing Education* 3: 869-878.
13. Xie, X., Brunner, N., Jensen, G., Albrechtsen, J., Gotthardsen, B., and Rygaard, J. 1992. *Clin. Exp. Metastasis* 10: 201-210.