Spontaneous Histiogenic Tumors of Epididymis Observed in B6C3F₁ Mice

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ABSTRACT. Four primary histiogenic tumors of the epididymis were discovered in 110 male B6C3F₁ mice used in a carcinogenicity study in our laboratory. From the results, all these tumors were considered to be spontaneous. Histologically, the tumors were characterized by compact growth of spindle to oval shaped histiocytic cells with or without a cleaved nucleus. Focal hemorrhage was present in three cases, and erythrophagocytosis occurred in such lesions. The tumors had some similar histological features of histiocytic sarcomas, which had been observed with high malignancy in other male and female animals. However, systemic growth and atypism of neoplastic cells found in the histiocytic sarcomas were not observed in these epididymal tumors. On the other hand, four histiogenic tumors of the uterus, with features characteristic of epididymal tumors, were observed in 106 female mice. The uterine histiogenic tumors were regarded as benign or precursor lesions of histiocytic sarcoma. Furthermore, it is suspected that histiocytic sarcoma arises from the epididymis.—**KEY WORDS:** B6C3F₁ mouse, epididymal tumor, histiocytic sarcoma, xanthofibroma.

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Sarcoma [6, 8], lipoma [6], hemangiosarcoma [6], leiomyosarcoma [10] and Leydig cell tumor [7] have been described as spontaneous tumors of the epididymis in mice. However, epididymal tumors are not common in mice. We identified four cases of primary epididymal tumors from 110 male mice (3.6%) used in a carcinogenicity study. The histological features of the neoplastic cells suggest that these tumors are derived from histiocytic cells. The tumors which were located in the epididymis resembled histiocytic sarcoma and were found in other male and female animals as a hematopoietic neoplasia. Therefore, we examined the histological characteristics of these histiogenic tumors in detail, and discuss the relationship between epididymal tumors and histiocytic sarcoma.

MATERIALS AND METHODS

Animal: Crj:B6C3F₁ mice (Specific pathogen-free, 4 weeks) were obtained from Charles River Japan Inc., on February 28, 1989. At the time of the final autopsy on November 2, 1990, they were approximately 90 weeks of age.

[Animal maintenance] The animals were housed, two per cage, in stainless-steel wire-mesh cages. The temperature of the room was maintained at 23±2°C and the humidity at 55±10%. Cool white fluorescent lighting was provided 12 hr daily. Diet (γ -ray exposed powder food, CE-2; CLEA Japan, Inc.) and water (ultrafiltrated tap water) were available *ad libitum*.

Design of the carcinogenicity study: The animals were divided into four experimental groups. Each group consisted of 50 males and 50 females. Totally 400 mice were used in this study. One of these groups was chemical-free (group 1, control group), and the others were chemically treated groups (group 2; low dose, group 3; middle dose and group 4; high dose). A test chemical was mixed in the above-mentioned diet and administered to the animals for

18 months. The animals were killed periodically one month after the final administration, and examined pathologically.

Pathological examinations: After necropsy, formalin-fixed paraffin-embedded hematoxylin-eosin stained tissue sections were prepared from 110 males and 106 females (groups 1 and 4, and animals which were dead or sacrificed during the test period in other two groups). Histological examination was carried out on the neoplastic lesions in the epididymis and compared with the histiocytic sarcomas or the uterine histiogenic tumors in the other male and female mice.

RESULTS

In the carcinogenicity study, there were no significant differences in the parameters indicating chemical carcinogenicities, between the control and high dose groups for either sex. No tumors were specifically observed in the chemically treated groups. These findings indicated that the test chemical was not carcinogenic. Therefore, the histiogenic tumors found in the treated groups were all considered to be spontaneous.

Incidence of histiogenic tumors (Table 1): Four histioge-

Table 1. Incidence of the histiogenic tumors in B6C3F₁ mice

Sex	Focal histiogenic tumor			Histiocytic sarcoma		
	D,S ^{a)}	PA ^{b)}	Total	D,S	PA	Total
Male	0/20 ^{c)} (0) ^{d)}	4/90 (4.4)	4/110 (3.6)	1/20 (5.0)	0/90 (0)	1/110 (0.9)
Female	0/15 (0)	4/91 (4.4)	4/106 (3.8)	2/15 (13.3)	0/91 (0)	2/106 (1.9)

a) Dead or sacrificed for moribund animals.

b) Animals at periodic autopsy.

c) Number of tumor-bearing animals/Number of histological examined animals.

d) (): %.

nic tumors located in the epididymis (epididymal histiogenic tumor) were observed in 110 males (3.6%) at the time of periodical autopsy. They did not exhibit any clinical signs. One of these tumors was found in group 1, and the other three in group 4. Only one case of histiocytic sarcoma in male animals (0.9%) was found in group 1. In 106 females, four histiogenic tumors were found in the uterus (uterine histiogenic tumor) (3.8%) at the time of periodical autopsy. Those, too, did not exhibit any clinical signs. One of these tumors was found in group 4, and the

others were found in group 1. There were two cases of histiocytic sarcoma in female animals (1.9%), being found in groups 2 and 3.

Histological features of histiogenic tumors (Table 2): Epididymal histiogenic tumors were nodular lesions, observed in the tail part of the unilateral epididymis. The largest one found in group 1 was yellowish-brown, slightly hemorrhagic, and $11 \times 8 \times 5$ mm in size. Histologically, the tumors were characterized by compact proliferation of neoplastic cells replacing most of the normal epididymal

Table 2. Histological characteristics of the histiogenic tumors

Animal No. (experimental group)	Tumor cell shape ^{a)}	FH ^{b)}	Erp ^{c)}	Other characteristics
Epididymal histiogenic tumor				
1(control group)	$S \cong O$	++	++	Capsular rupture
2(high dose group)	$S \gg O$	±	±	—
3(high dose group)	$S \gg O$	—	—	—
4(high dose group)	$S \cong O$	±	±	—
Uterine histiogenic tumor				
5(control group)	$S < O$	±	—	—
6(control group)	$S \cong O, G$	—	—	—
7(control group)	$S \cong O$	+	+	—
8(high dose group)	O, G	—	—	—
Histiocytic sarcoma				
9(male control group)	$S \cong O, G$	++	++	Systemic growth
10(female low dose group)	$S \cong O$	++	++	Systemic growth
11(female middle dose group)	$S \cong O, G$	+	+	Systemic growth

a) The shape of neoplastic cells observed in tumor tissues (S: spindle cell, O: oval cell) and relative proportion of them ($\cong$, $\gg$, $<$), and appearance of giant cell (G).

b) Focal hemorrhage and c) erythrophagocytosis were found in large (++) to very small (±) area of the tumor, and not observed (—).

Fig. 1. Epididymal histiogenic tumor in an animal of control group. The tumor is characterized by compact proliferation of neoplastic cells. Focal hemorrhage (●) and capsular rupture (⊙) are observed. H.E. $\times 16$.

Fig. 2. A part of Fig. 1. Neoplastic cells show morphologic variation, from round or oval to spindle or fusiform. H.E. $\times 211$.

tissue (Fig. 1). The neoplastic cells were pleomorphic varying from round or oval to spindle or fusiform. The relative proportion of these two cell types varied from case to case (Fig. 2). The tumor cells had distinct eosinophilic cytoplasm, similar to foamy cells, in some places (Fig. 3).

Fig. 3. High-power view of a part of Fig. 1. Oval neoplastic cells have abundant, distinctly eosinophilic cytoplasm. H.E. $\times 634$.

The nucleus in most neoplastic cells had a sharp cleavage. Focal hemorrhage was present in three tumors with erythrophagocytosis in such foci (Fig. 4). Hemorrhage was more extensive in the largest tumor as compared to the other tumors (Fig. 5). Invasive growth of the neoplastic

Fig. 4. A part of Fig. 1. Erythrophagocytosis occurred in a focal hemorrhage area. H.E. $\times 557$.

Fig. 5. A part of Fig. 1. The hemorrhagic area of this tumor is larger than in other epididymal histiogenic tumors. H.E. $\times 95$.

Fig. 6. High-power view of the part with capsular rupture of Fig. 1. H.E. $\times 85$.

Fig. 7. Neoplastic cells of uterine histiocytic tumor. Histologically, they are similar to the cells of epididymal histiocytic tumors. H.E. $\times 634$.

Fig. 8. Multinucleated giant cells appeared in a uterine histiocytic tumor. H.E. $\times 221$.

Fig. 9. Neoplastic cells of histiocytic sarcoma (liver). They show a great variation in size and shape, and atypism, having the abundant, distinctly eosinophilic cytoplasm. Histologically, these cells resemble the cells of epididymal or uterine histiocytic tumors except for marked malignancy. H. E. $\times 634$.

Fig. 10. Focal hemorrhage and erythrophagocytosis observed in histiocytic sarcoma (liver). These histological findings are common with the three cases of epididymal histiocytic tumor. H.E. $\times 557$.

cells in this case resulted in rupture of the capsule (Fig. 6). Giant cells were not observed in the epididymal lesions.

In females, all the uterine histiogenic tumors were solitary nodular lesions, within a range of 10 mm in diameter. They were observed either in the cervix or horn of the uterus. Histologically, they were very similar to the epididymal histiogenic tumors, and proliferation of histiocytic type cells with or without a cleaved nucleus was observed (Fig. 7). Focal hemorrhage was observed in two cases, and erythrophagocytosis occurred in one of them. Numerous multinucleated giant cells appeared in two cases (Fig. 8).

Histiocytic sarcomas were observed in three animals as a systemic hematopoietic neoplasia. Primary organs were the liver in the male, and the liver or uterus in females. Histologically, they were characterized by nodular and infiltrative proliferation of histiocytic type cells with marked variation in the size and shape. The basic cell had abundant, distinctly eosinophilic cytoplasm with or without nuclear cleavage (Fig. 9). The nucleus was U- or ring-shaped in some places. Focal hemorrhage was commonly observed in the lesions, and erythrophagocytosis occurred principally in the liver (Fig. 10). The histiocytic sarcomas histologically resembled the epididymal or uterine histiogenic tumors except for their characteristics of high malignancy and severe atypism of the neoplastic cells.

DISCUSSION

Although epididymal neoplasia is rare, we identified four primary histiogenic tumors of the epididymis in 110 male B6C3F₁ mice. Such a high incidence suggests that the animals in the lot had a predisposition to this neoplasia.

The epididymal histiogenic tumors must be discriminated histologically from spermatic granuloma and Leydig cell tumor. Spermatic granuloma of the epididymis shows a central dense mass of entangled spermatozoa, surrounded by radially arranged epithelioid, with multinucleated giant cells, and vascular connective tissue containing dense infiltrates of inflammatory cells [1, 5]. None of these features were noted in our cases, thus distinguishing them from spermatic granuloma. In Leydig cell tumor of the epididymis, neoplastic cells are arranged in cords or packets separated by a delicate fibrovascular stroma [7]. However, the tumors in our cases showed compact growth of the cells without stroma. The histiocytic characters of the cells in monotonous proliferation suggest that the lesions observed here were neoplasia arising from a histiocytic cell.

Histiocytic sarcoma that arises principally from the liver in male mice, and liver or uterus in female mice, is a malignant neoplasm of the hematopoietic system arising from the histiocyte [3, 4, 9]. We found three cases of histiocytic sarcoma in this study. These tumor cells with or without a cleaved nucleus resembled the histiocyte and the shape of the cells varied from oval to spindle. These cells showed solid proliferation without stroma in the tumor tissues. These histological features were common to the

epididymal histiogenic tumors. Additionally, focal hemorrhage with erythrophagocytosis, which is one of histological characteristics of histiocytic sarcoma [3, 4], was found in three cases of epididymal histiogenic tumors. Therefore, it was suggested that epididymal histiogenic tumor was similar to histiocytic sarcoma. However, systemic growth of the tumor and severe atypism of neoplastic cells, seen in histiocytic sarcoma, were not observed in the epididymal histiogenic tumors.

On the other hand, the histiocytic sarcoma is frequently localized in the uterus of female mice [2]. In our observation, four histiogenic tumors were located in the uterus in addition to the histiocytic sarcomas. These uterine histiogenic tumors were regarded as benign or precursor lesions of histiocytic sarcoma, because the uterus is one of the primary sites of predilection to histiocytic sarcoma. The epididymal histiogenic tumors were histologically similar to the uterine tumors except for the appearance of giant cells. Therefore, it is reasonable to regard the epididymal histiogenic tumors as benign or precursor lesions of histiocytic sarcoma. We suspect that histiocytic sarcoma arises from the epididymis. It is uncertain whether or not the largest tumor of the epididymis is benign, because histologically it showed some malignancy. This tumor had an extensive hemorrhagic lesion with erythrophagocytosis and capsular rupture. Therefore, in a broad sense, it may be appropriate to name this case as a histiocytic sarcoma. We decided to name the other cases of epididymal histiogenic tumor as xanthofibroma, due to their indistinct malignancy. However, it is not certain whether they are essentially different. They could be potentially malignant.

In conclusion, the epididymal histiogenic tumors that we found in B6C3F₁ mice, appeared to be closely related to histiocytic sarcoma. However, we could not demonstrate the cell of origin and the characteristics except for the histological pattern of the tumors.

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