



NOTE

Pathology

Jejunal fibroplasia in a rat

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ABSTRACT. A jejunal nodular mass was identified in an aging rat. Histologically, the boundaries between the lesion and surrounding normal tissue as well as between the inner circular muscle and outer longitudinal muscle were indistinct. The lesion consisted of abundant eosinophilic matrix and cells with a large round to oval nucleus and indistinct cytoplasm. There was no characteristic proliferating pattern, nuclear polymorphism and a low mitotic figure count. Masson's trichrome stain revealed that the intestinal smooth muscles were replaced by the abundant collagen fiber. Immunohistochemistry revealed that the cells with a large round to oval nucleus were labeled with anti-vimentin antibody and not with anti- α smooth muscle actin antibody, suggesting that these cells were fibroblasts. The mass was diagnosed as jejunal fibroplasia.

KEY WORDS: fibroplasia, jejunum, rat

In the gastrointestinal tract, feline gastrointestinal eosinophilic sclerosing fibroplasia (FGESF) is known as a disease forming a mass caused by excessive collagen fibers. This disease has been reported in cats and pumas (*Puma concolor*) [2–4, 9]. The etiology of this disease is the infection (bacteria, fungi and nematodes), foreign body or unknown [2–4, 7, 9]. Macroscopically, the lesion is observed as an ulcerated intramural mass at the pyloric sphincter and the ileoceocolic junction [2–4, 9]. Microscopically, all lesions include a very characteristic trabecular pattern of dense collagen with proliferation of the reactive large myofibroblasts. Many lesions also have numerous eosinophils and mast cells [2, 3, 7, 9]. In cats, the term 'FGESF' is considered appropriate based on these microscopic characteristics [2]. In other animals including humans, to our knowledge, there are no descriptions of a lesion caused by the replacement of the intestinal smooth muscle layer by the excessive collagen fiber in the gastrointestinal tract.

A 110-week-old female Sprague-Dawley rat purchased from Charles River Japan Inc. (Yokohama, Japan) at five-week-old was part of a group being kept to obtain background data for a 2-year carcinogenicity study. The animal was housed in an aluminum cage and cared for according to the principles outlined in the guidelines for the care and use of laboratory animals issued by the Japanese Association for Laboratory Animal Science and our laboratory. The animal was fed a basic diet (CRF-1: Oriental Yeast Co., Ltd., Tokyo, Japan) and allowed free access to tap water. Necropsy was performed at the end of keeping period. Gross examination revealed a white nodular mass (approximately 5 mm in diameter) in the jejunum at the opposite side of the mesentery. There was no adhesion of the jejunum to surrounding tissues. The cut surface of the jejunal mass was white. There were many macroscopic changes in the whole body, however, no changes considered to be associated with the jejunal mass were observed.

Samples from a range of organs were fixed in 10% neutral buffered formalin and were embedded in paraffin wax. Sections were stained with hematoxylin and eosin (HE), Masson's trichrome (MT), Watanabe's silver, and eosin and methylene blue (EMB) stains. Microscopic examination revealed the lesion was located from submucosa to serosa at the opposite side of the mesentery. The boundaries between the lesion and surrounding normal tissue as well as between the inner circular muscle and outer longitudinal muscle were indistinct (Figs. 1 and 2; Supplementary Figs. 1 and 3). The lesion consisted of abundant eosinophilic matrix and cells with indistinct cytoplasm, which were embedded in the eosinophilic matrix (Figs. 3 and 4). The nuclei were large, oval with finely stippled chromatin, and the nucleoli were found to be one or indistinct (Fig. 4). There was no characteristic proliferating pattern, nuclear polymorphism, and one or less mitotic figures per high power field ($\times 400$). MT stain revealed that the smooth muscle layers stained red were replaced by the abundant collagen fiber stained blue (Figs. 2 and 5; Supplementary Figs. 2 and 4). Watanabe's silver stain revealed that argyrophilic fibers stained black surrounding the smooth muscle cells disappeared in the lesion (Fig. 6). EMB stain revealed that a few eosinophils and mast cells were infiltrated into the lesion (Fig. 7). In addition, thickening of the mucularis mucosae and hypertrophy of the villi by the infiltration of lymphocytes and plasma cells at the opposite side of the mesentery were also observed. There was no ulceration in the mucosa or focal necrosis, granulation tissue formation, infectious agents (bacteria, fungi and parasite) or foreign bodies in the lesion.

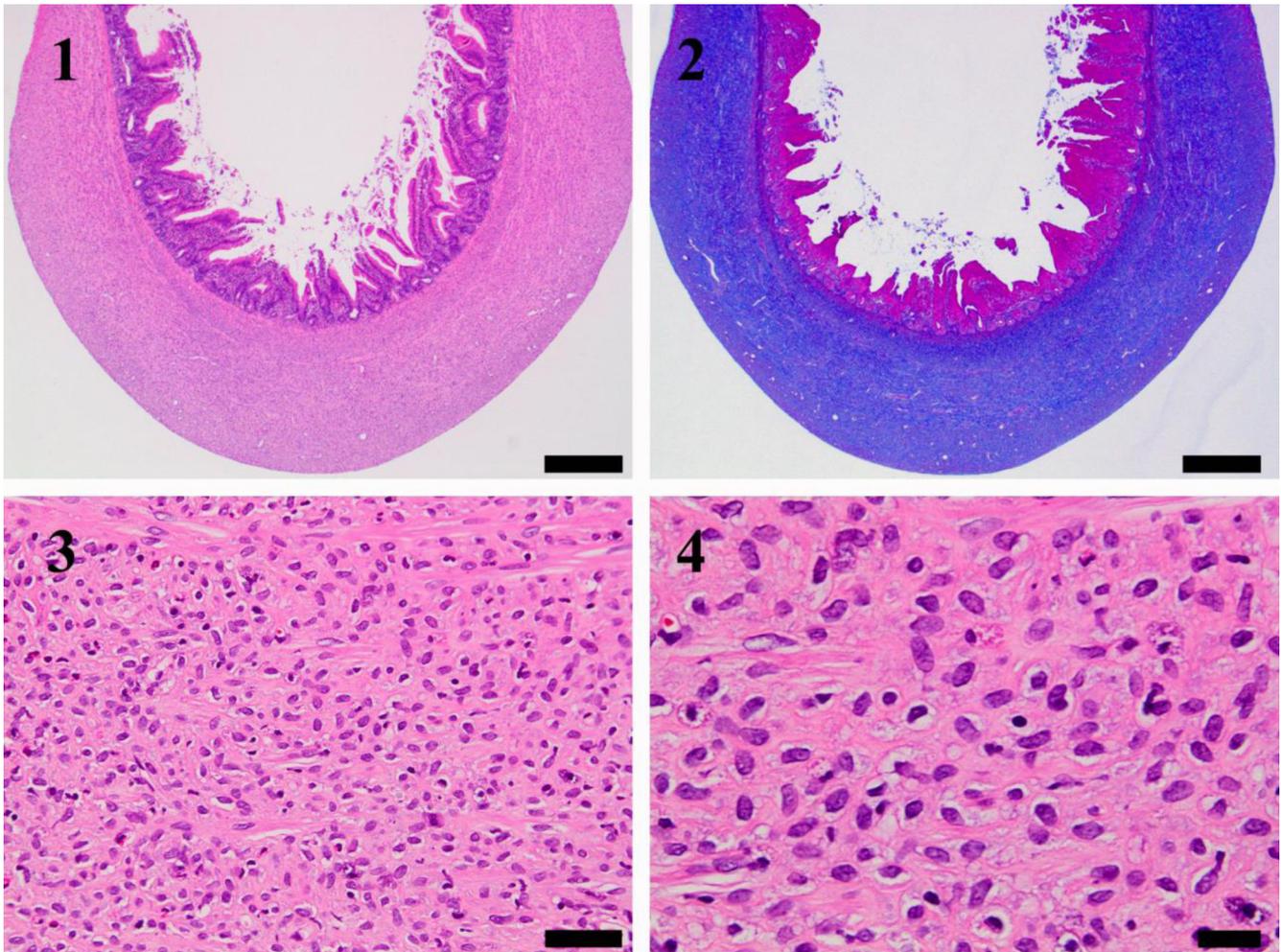
Immunohistochemistry (IHC) was performed using a commercial kit (Vectastain Elite ABC Kit, Vector Laboratories Inc., Burlingame, CA, U.S.A.) with biotinylated goat anti- mouse and anti- rabbit IgG (Vector Laboratories Inc.) and avidin-biotin-

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- Fig. 1.** The lesion is located from submucosa to serosa at the opposite side of the mesentery. The boundary between the inner circular muscle and outer longitudinal muscle is indistinct. HE. Bar, 500 μ m.
- Fig. 2.** The smooth muscle layers stained red of the jejunum are replaced by the abundant collagen fiber stained blue. MT. Bar, 500 μ m.
- Fig. 3.** The lesion consists of abundant eosinophilic matrix and cells with indistinct cytoplasm, which are embedded in the eosinophilic matrix. There is no characteristic proliferating pattern. HE. Bar, 50 μ m.
- Fig. 4.** The cells with a large, oval and chromatin-finely stippled nucleus and a small, irregular and chromatin-rich nucleus are observed in the lesion. There is no nuclear polymorphism or mitotic figures per high power field ($\times 400$). HE. Bar, 20 μ m.

peroxidase conjugate. Mouse monoclonal anti-porcine vimentin antibody (Dako, Carpinteria, CA, U.S.A.), rabbit monoclonal anti-human alpha smooth muscle actin (α SMA) antibody (Abcam, Tokyo, Japan) and rabbit polyclonal anti-rat ionized calcium binding adapter molecule 1 (Iba1) antibody (Wako Pure Chemical Industries, Osaka, Japan) were used as the primary antibodies. Negative controls were performed by omitting the primary antibody. IHC revealed that the cells with a large round to oval nucleus were labeled with anti-vimentin antibody (Fig. 8) and not with anti- α SMA antibody (Fig. 9), suggesting that these cells were fibroblasts. On the other hand, many cells with a small, irregular and chromatin-rich nucleus were also observed in the lesion (Fig. 4). IHC revealed that the cells with a small nucleus were labeled with anti-Iba1 antibody (Fig. 10), suggesting that these cells were macrophages.

It is considered that fibrogenic factors, such as major basic protein, transforming growth factor (TGF)- β , interleukin (IL)-1 β and IL-13 secreted by infiltrated eosinophils and mast cells into the lesion, are related to the proliferation of the fibroblasts and the formation of the sclerosing fibers as the pathogenesis of the FGESF [1-4, 7, 9]. In present case, many macrophages labeled with anti-Iba1 antibody and a few eosinophils and mast cells were observed compared with that in the lesion of FGESF. There were no descriptions of a relation of macrophage to the formation of FGESF lesions; however, it is known that fibrogenic factors, such as TGF- β and monocyte chemoattractant protein-1 (MCP-1), are also secreted by macrophages [11]. TGF- β 1, a well-known fibrogenic factor, is produced mainly by infiltrated macrophages and related to fibrosis in the intestinal tract [10]. MCP-1, a chemokine associated with the migration of macrophages, is related to fibrosis in the peritoneum [6]. Therefore, it may be possible

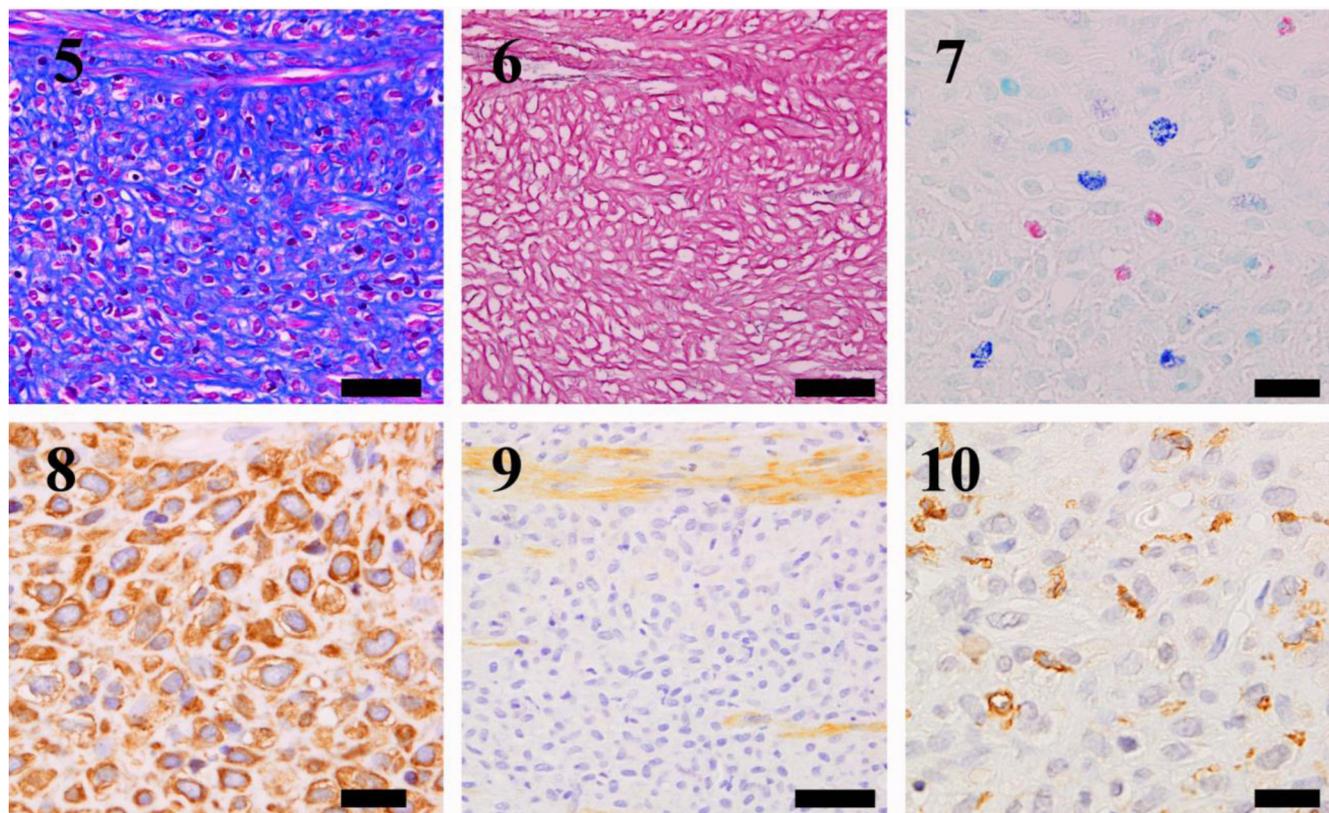


Fig. 5. Most of the smooth muscles are replaced by the abundant collagen fiber stained blue, and there are a few residual inner circular smooth muscles stained red. MT. Bar, 50 μ m.

Fig. 6. Argyrophilic fibers stained black surrounding the smooth muscle cells disappear in the lesion. Watanabe's Silver. Bar, 50 μ m.

Fig. 7. A few eosinophils with red granules and mast cells with blue granules are infiltrated into the lesion. EMB. Bar, 20 μ m.

Fig. 8. The cells with a large round to oval nucleus are labeled with anti-vimentin antibody. IHC. Bar, 20 μ m.

Fig. 9. The cells with a large round to oval nucleus are not labeled with anti- α SMA antibody. Most of the smooth muscles disappear, and a few residual inner circular smooth muscles are labeled with anti- α SMA antibody. IHC. Bar, 50 μ m.

Fig. 10. The cells with a small, irregular and chromatin-rich nucleus are labeled with anti-Iba1 antibody. IHC. Bar, 20 μ m.

that the fibrogenic factors were secreted not only by the eosinophils and mast cells but also by macrophages, and could be related to the proliferation of the fibroblasts and the deposition of the extracellular matrix and collagen fibers.

Pathogenesis of this lesion may be similar to that of FGESF on the basis of the possible relation of the fibrogenic factor. However, the histologic feature of this lesion did not match to that of FGESF. In pumas, the name 'sclerosing fibroplasia' is proposed, because infiltration of a few eosinophils into the lesion was observed as opposed to that of the FGESF [3]. In present case, the infiltration of eosinophils and mast cells was mild, and there was no formation of the trabecular sclerosing collagen fiber. Therefore, we diagnosed this lesion as 'fibroplasia' in the rat jejunum.

To our knowledge, the definition of the term 'fibroplasia' in the small intestine has not been clarified properly. In the small intestine, it is very difficult to define and differentiate fibrosis and fibroplasia as we consider it possible that fibroplasia represents different histologic futures depending on the tissues and organs in which the term is used. In the skin, fibroplasia and fibrosis are defined as sequential reactions of wound healing. Fibroplasia is often used synonymously with granulation tissue, and fibrosis is a late stage of fibroplasia [12]. When we follow the definition of this field, the lesion in this case is considered to be fibrosis. On the other hand, in the medium-sized muscular artery, fibroplasia of the intima, media and adventitia is described as fibromuscular dysplasia [5]. In this disease, medial fibroplasia is classified into one of the medial dysplasia and defined as thickening of the media caused by the replacement of the medial smooth muscle by collagen [8]. Therefore, it is reasonable to define that fibroplasia is the lesion that the original constituents of the organs and tissues are replaced by excessive collagen. When we follow the definition of this field, the lesion in this case is considered to be fibroplasia. Although there is a difference between the digestive system and the cardiovascular system, we diagnosed the lesion in this case as fibroplasia from the similarities of the lesion in that the smooth muscle is replaced by collagen in the tubular tissue, in which the smooth muscle is one of the main components.

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