Superficial Eosinophilic Gastritis in Laboratory Beagle Dogs Attributable Probably to Diet

Isao NARAMA*, Junji KURODA, Mariko NAGATANI, Kouji MIURA, Toshiyuki KATOH, and Minoru TSUCHITANI

Hamamatsu Seigiken Research Laboratory, Co., Aoicho 95-10, Hamamatsu, Shizuoka, 433, Japan

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ABSTRACT. Stomach tissues from 222 beagle dogs, which were used for toxicological studies, were examined histopathologically. Out of 190 dogs fed the same diet, 185 had gastric lesions, whereas all of 32 dogs fed another diet were free from them. The gastric lesions were noted as an accumulation of eosinophils, infiltration of inflammatory cells including lymphocytes, plasma cells and histiocytes in the gastric lamina propria, formation of foreign body type multinucleated giant cells, focal desquamation and papillary proliferation of mucosal epithelium. The lesions were confined to the superficial layer of the antral and pyloric mucosa, and neither dysplasia nor clinical signs were seen. Multinucleated giant cells often contained amphophilic foreign bodies of varying appearance with inconsistent reaction for periodic acid-Schiff (PAS), but no definite causal agents were detected.—**KEY WORDS:** antral mucosa, diet, foreign body, giant cell, pyloric mucosa.

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Stomach inflammatory lesions are thought to be very common in many species of domestic animals and man. However, little attention has been paid to the lesions of superficial gastritis, because of occult clinical signs and obscure origins. Lack of well-defined histologic criteria for the diagnosis of gastritis seems to be another reason for the rather small number of reports on gastritis in domestic and laboratory animals [10]. Some special types of gastritis have been reported in animals, as well as some cases with well-defined causes [13-15, 20, 22].

The present report describes a high frequency of superficial eosinophilic gastritis in laboratory beagle dogs fed a standard commercial diet.

MATERIALS AND METHODS

Stomach tissues from 222 beagle dogs

* CORRESPONDENCE TO: NARAMA, I., Institute of Drug Safety, Setsunan University, 45-1 Nagaotoge-cho, Hirakata, Osaka, 573-01, Japan.

were subjected to histological examination. All animals were used in toxicity studies for five drugs. These developmental drugs were of various chemical structures with different pharmacological effects, and administered orally or intramuscularly into animals (Table 1). One hundred and forty-four animals (test groups A, B, C and F) were purchased from two breeders in Japan (C.S.K. Farm, Nagano and Fuji Animal Farm, Yamanashi), and the remaining 78 animals (test groups D and E) were from a breeder in the United States (Marshall Research Animals, NY). All animals were housed individually in aluminum cages, in animal rooms conditioned at $24 \pm 2^\circ\text{C}$ temperature, with $50 \pm 15\%$ relative humidity, under 12 hr of artificial light. After their arrival, all dogs but those in group F were supplied with 300 g/day of a commercial diet (Labostock D, Nihon Nosan, Japan) and chlorinated tap water *ad libitum* for an acclimation period of about 30 days and following test periods of varying duration. Thirty-two dogs in group F were fed another

Table 1. Incidence of stomach lesions in beagle dogs

Group	(test substance)	Administration route	Test period	No. of positive/Total (percentage)
A	(artificial nutritional substance)	P.O. ^{a)}	125 days	32/36 (88.9)
B	(analgesic)	I.M. ^{b)}	70 days	38/38 (100)
C	(analgesic = same as B)	I.M.	215 days	38/38 (100)
D	(adrenoreceptor β -blocker)	P.O.	70 days	38/38 (100)
E	(antiadenoviral drug)	P.O.	215 days	39/40 (97.5)
F	(adrenoreceptor β -blocker)	P.O.	125 days	0/32 (0)

a) Peroral.

b) Intramuscular.

commercial diet (DS, Oriental Yeast, Japan) under the same conditions. Both diets contained corn, meat meal, soybean, wheat powder, skim milk, beet pulp, fish meal, powdered bone, soybean oil, fat, refined inorganic minerals and vitamins as essential ingredients, although there were no data available for the exact mixing rate of both diets. In the test for the substance A, some animals in the highest dosage group were not allowed to eat any diet for about 1 month prior to death, and instead, they were administered an excess amount of artificial nutrient (30 g/kg/day). All animals were examined daily for clinical signs. Examinations on hematology, serum biochemistry and electrocardiogram were performed at various intervals. There were no abnormal findings relating to gastric lesions.

The period of exposure to Labostock D ranged from about 100 days in test groups B and D, to about 245 days in C and E, with about 30 days acclimation and various test periods. At the end of various test periods, all animals were anesthetized with ketamine, killed by bleeding from the carotid artery, and necropsied.

Alimentary tracts, including the stomach were removed and immediately immersed in a 10% formalin solution. Stomachs were incised along the great curvature, washed in a 10% formalin solution and stretched on a flat board. Gastric tissues for microscopic examination were sampled from six repre-

sentative sites; two tissue blocks from each of the ventricular body, pyloric antrum, and pylorus of all dogs, as well as some other parts of the stomach in many cases. All tissue blocks were trimmed, washed in tap water, defatted and dehydrated in an automated processor, and embedded in paraffin. Sections were cut at 5 μ m and stained with hematoxylin and eosin (H & E). Some representative sections were also stained with periodic acid-Schiff (PAS), Ziehl-Neelsen for acid-fast bacilli, Carbol-Thionin and Hucker-Conn's Gram stain for non-specific bacteria, Grocott's methenamine silver method for fungi, and von Kossa's method for calcium.

Tissue samples for electron microscopy were taken from pyloric mucosa of two cases from group E. They were fixed in 2% glutaraldehyde for 2 hr, washed in phosphate buffer, post-fixed in buffered 1% osmium tetroxide for 1 hr, dehydrated in an ethanol series, and embedded in resin. Ultra-thin sections were stained with lead citrate and uranyl acetate and examined by an electron microscope (JEOL, 100CX-II).

RESULTS

No abnormal findings were detected by gross examination of the gastric mucosa in any case. Microscopically, the lesions were confined to the antrum and a narrow circular zone of the ventricular body adjacent to

the antrum. The microscopic changes were milder in mucosa remote from the pylorus and were never seen in the major part of the ventricular body, fundus, or cardia. However, there were no significant differences in the extent and degree of the gastric lesion between the control and treated animals on test, and among the animals of various test substances or test periods.

The gastric lesions consisted of an accumulation of eosinophils just beneath the epithelium, infiltrations of mononuclear inflammatory cells, including lymphocytes, plasma cells and histiocytes into the lamina propria, formation of multinucleated giant cells phagocytizing foreign body in the lamina propria, focal desquamation of mucosal epithelium, and papillary proliferation of epithelial cells in pyloric glands (Table 2). In the affected gastric mucosa, the foveola showed irregular branching, distortion, and elongation (Fig. 1). Lymphocytes, plasma cells, and histiocytes were scattered throughout the whole lamina propria of the affected mucosa, although marked inflammatory edema, hemorrhage, or degeneration of surface epithelial cells were not seen. At some places, large numbers of eosinophils aggregated just beneath the surface epithelial cells (Fig. 2). An eosinophilic amorphous substance was found at the center of accumulated eosinophils (Fig. 3) and occasionally permeated the surface epithelial cells. Microerosions were frequently noted in these areas, and

degenerated eosinophils or other inflammatory cells erupted into the gastric foveola from the erosive site (Fig. 4). Papillary proliferation of the epithelial cells was occasionally seen in the pyloric gland or deep gastric foveola (Fig. 5), indicative of reactive hyperplastic regeneration following the desquamation of epithelial cells. The severity of the gastric lesions was variable in each case and unrelated to the length of the test period.

Multinucleated foreign body giant cells were also scattered throughout the lamina propria (Fig. 1). Some giant cells were found just beneath and among the epithelial cells (Fig. 6). Foreign bodies, probably of exogenous derivation, of granular, amorphous or fibrillar appearances, with amphophilia were seen in almost all giant cells (Figs. 6 and 7). Some foreign bodies showed similar appearances to the debris of dietary plant fibers. Many giant cells were degenerative and contained pyknotic nuclei. Some foreign bodies in the giant cells stained faintly to strongly positive with PAS reaction, although the positive area had no definite border or shape. Some foreign bodies took on the appearance of calcifying spherules, but they were negative for calcium by von Kossa's method. Some of the engulfed foreign bodies were anisotropic. No meaningful results were obtained from repeated staining by Ziehl-Neelsen's method for acid-fast bacilli, by carbolfuchsin or Hucker-Conn's method for bacte-

Table 2. Microscopic findings of gastric lesions in beagle dogs fed two different diets

Diet	Severity ^{a)}	Accumulation of eosinophils	Desquamation of epithelial cells	Giant cell formation	Papillary proliferation of foveola epithelium	Inflammatory cell infiltration
Labostock D	—	51/190	37/190	5/190	144/190	2/190
	+	52/190	60/190	64/190	46/190	57/190
	++	74/190	74/190	114/190	0/190	113/190
	+++	13/190	19/190	7/190	0/190	18/190
DS	—	32/32	32/32	32/32	32/32	32/32

a) — ; no lesion found, + ; mild, ++ ; moderate, +++ ; severe

Fig. 1. Irregular branching, distortion, and elongation of gastric foveola in the pyloric antrum. Many foreign body giant cells in the lamina propria (arrows). H & E. $\times 50$.

Fig. 2. Accumulation of eosinophils beneath epithelium. H & E. $\times 260$.

Fig. 3. Eosinophilic amorphous (proteinous) substance formed in the center of accumulated eosinophils. H & E. $\times 260$.

Fig. 4. Inflammatory cells exuding into the gastric foveola from a microerosion. H & E. $\times 130$.

Fig. 6. Multinucleated giant cell with pyknotic nuclei beneath epithelium, containing foreign bodies with fibrillar appearance. H & E. $\times 520$.

Fig. 5. Papillary proliferation of epithelial cells in the pyloric gland. H & E. $\times 260$.

rial agents. Fungi were also absent by Grocott's methenamine silver method. These changes were diagnosed as superficial eosinophilic gastritis and seen in 185 out of 190 dogs fed the same diet of "Labostock D". Only 4 dogs in test group A that were not fed the pelleted diet for about 1 month before death, one dog in test group E which was fed the diet, and 32 dogs fed another diet, were free of these gastric lesions.

Electron-microscopically, many macrophages and multinucleated giant cells characterized by many lysosomal vesicles and well-developed interlocking processes were seen in the gastric mucosa. Some lysosomes contained lamellar bodies and amorphous substances. Multinucleated giant cells or macrophages contained larger phagosomes which seemed to be formed by several lysosomal vesicles, including electron lucent homogeneous materials, lamellar bodies, electron opaque deposits or

Fig. 7. Two giant cells engulfing foreign bodies in the lamina propria. The nuclei of a giant cell are pyknotic. H & E. $\times 520$.

Fig. 8. Collection of glycogen granules and homogeneous materials surrounded by myelin lamellae in phagosomes. $\times 26,600$.

membranous structures in their limiting membrane. Some phagosomes showed collection of glycogen granules which were thought to correspond to PAS positive substances by light microscopy and homogeneous materials surrounded by myelin lamellae (Fig. 8). Irregular-shaped large aggregates of low-density electron-opaque fine granules and membranous structures were thought to correspond to the large foreign bodies seen by light microscopy in the giant cells (Fig. 9). Some multinucleated giant cells had degenerative nuclei and cytoplasmic organelles of higher electron density than those of adjacent macrophages.

DISCUSSION

Histopathologic features of the gastric lesions in the present dogs are thought to be

similar to those of eosinophilic gastroenteritis or chronic eosinophilic enteritis in dogs, cats, and horses [4-6, 16, 17], because eosinophils were predominant among the infiltrated inflammatory cells. However, the abnormal clinical signs such as repeated vomiting, persistent diarrhea and weight loss, which are reported in some affected animals with this disease [4, 5, 17], were not seen in any of our beagle dogs. Hypoalbuminemia [5] or circulating eosinophilia [5, 17], which are often reported in affected dogs, were also not seen in our dogs despite repeated hematological and blood chemical examination. It is unlikely that this silent gastric disorder belongs to a previously described syndrome, because no clinical abnormality has ever been detected in a large number of dogs by repeated examination. Macroscopic changes, with swelling of the affected mucosa, ulceration, erosion,

Fig. 9. Irregularly-shaped large debris (D) of low density containing electron-dense granules with many phagosomes (arrows) in a degenerated giant cell. Nuclei and cytoplasm of the giant cell are more electron-dense than those of adjacent macrophages. $\times 3,900$.

enlargement of mesenteric lymph node, or widespread invasion of inflammatory changes reaching the serosa in microscopic examination [4, 5, 17], as described by various authors, were not encountered in our dogs.

The accumulation of eosinophilic amorphous substance just beneath the epithelium might play an important role for subsequent desquamation and regenerative hyperplasia. The origin of the eosinophilic amorphous substance is obscure. The subepithelial accumulation of eosinophils suggested a close association of eosinophils with this substance, but it is uncertain whether the chemotactic action of this substance accumulated the eosinophils or the released eosinophilic granules (or debris of the degenerated eosinophils) formed this substance with tissue or hematogenous protein.

The origin and role of foreign bodies in

the giant cells cannot be confirmed. However, the absence of definite causative bacteria [12, 18, 19, 21] or fungi [14, 15], which are known to evoke histiocytic accumulation and multinucleated giant cells, is thought to be evidence for non-involvement of an infectious agent.

Infiltration of eosinophils might suggest a parasitic infestation as a cause of the gastric lesions. The stomach worm such as *Physaloptera* spp., and visceral larva migrans of *Toxocara* spp. have been reported to evoke eosinophilic gastritis in dogs [5]. However, the size of the cuticle of these parasites is much larger than the observed "foreign bodies". Indeed, lung worm was the only parasite found in any of our dogs, forming granulomatous lesions in the mesenteric lymph nodes or occupying alveolar spaces in the lung without tissue reaction.

Coexistence of acute desquamative

change with papillary proliferation of the epithelium suggested that there were long-standing irritative effects on the gastric mucosa in the dogs examined. On the other hand, this irritative action might be mild and minimal, because inflammatory changes were restricted to the superficial layer of the mucosa and failed to show progressive characteristics with dysplasia. This is another difference from the eosinophilic gastritis reported previously in dogs [4, 5, 17].

The distribution of animals with gastric lesions strongly suggests that these gastric lesions are attributable to the animal diet, since experimental conditions were constant for all the groups, except for the type/source of diet. The exact cause of gastritis in this report, particularly of accumulation of eosinophils just beneath the gastric mucosa and of subsequent desquamation of epithelial cells still remains unclear. Recently, some authors reported gizzard erosion in chicks induced by feeding of certain batch of fish meal [8, 9, 11]. In these reports, a high concentration of histamine, which was thought to be produced by inadequate treatment of fish materials [2, 3], may have caused the erosions and ulcerations [7]. Some immunological mechanism may also cause the accumulation of eosinophils, but there is no conclusive evidence of any local or generalized hypersensitivity [1].

Commercial diets served to the beagle dogs in this study contained 5–20% fish meal. However, the sources and batch numbers for other raw materials in the diet were not available. Therefore, the exact cause of the superficial gastritis, including its immunological mechanism in our dogs, could not be determined. It is possible that other materials contained in the diet might evoke such changes. Further investigations are needed to clarify the pathogenesis of these gastric lesions. Pathologists who use dogs as experimental animals for the assess-

ment of the gastro-intestinal effect of various chemicals should pay particular attention to the changes described in the present study.

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要 約

実験用ビーグル犬における飼料性と思われる好酸球性表層性胃炎の頻発：奈良間 功・黒田淳二・永谷真理子・三浦浩二・加藤利幸・土谷 稔(株)生物科学技術研究所)——毒性試験に用いられた222頭のビーグル犬の胃を病理組織学的に検索したところ、同一の飼料を与えられた190頭中185頭に胃病変が認められた。胃病変は粘膜固有層における好酸球の集積，リンパ球・形質細胞・組織球を含む炎性細胞の浸潤，異物型多核巨細胞の形成，粘膜上皮の限局性剝離および乳頭状増殖から構成されていた。病変は幽門前庭部および幽門腺部粘膜の表層に限局し，結合組織成分の増加は認められなかった。多核巨細胞はしばしば様々な形態の異物を貪食していたが，病変の原因とみなされる微生物は証明されなかった。病変を有する例に臨床症状は認められなかった。なお，他の飼料を与えた32頭のビーグル犬には，本病変は認められなかった。