

**NOTE Pathology****Thymoma and Multiple Thymic Cysts in a Dog with Acquired Myasthenia Gravis**

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**ABSTRACT.** An anterior mediastinal cystic lesion in an 11-year-old mongrel dog was examined. The dog showed dysbasia and vomiting due to megaesophagus, and anterior mediastinal round mass lesion, approximately 35 mm in diameter, was found by X ray. Based on clinical examinations, the dog was diagnosed as acquired myasthenia gravis and was successfully controlled by anticholinesterase treatment for approximately 4 months. The dog died of thermic stroke and was necropsied. Grossly, fatty tissues with cysts containing yellowish fluid and white nodules were found in the anterior mediastinal area. Histopathologically, multiple cysts, neoplastic tissues, and atrophic thymus were found within the examined tissues. The cysts were lined by thin wall consisting of ciliated long cuboidal and non-ciliated round cells and were filled with eosinophilic colloidal fluid. Some extended cysts contained neoplastic foci within their lumen and walls. The neoplastic tissues consisted of mixed population of large epithelial cells with abundant clear cytoplasm and large oval nuclei, and lymphocytes. Immunohistochemically, proliferating epithelial cells were intensely positive for keratin and cytokeratin, and more than half number of infiltrating lymphocytes were intensely positive for CD3 suggesting T cells. All these findings indicate the neoplastic lesion is thymoma and multiple cysts are considered as thymic or brachial cleft cysts.

**KEY WORDS:** canine, thymic cyst, thymoma.

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Thymomas are rather common tumors in the anterior mediastinal area of dogs [2, 3, 5, 7, 16], and some are associated with acquired myasthenia gravis and autoimmune paraneoplastic syndrome [6, 8, 10, 12, 17, 18, 22]. The clinical and pathological features of thymomas in domestic animals are well documented. Histopathologically, thymomas are characterized by mixed population of thymic epithelial cells and lymphocytes, and can be classified into epithelial dominant, lymphocyte dominant, and mixed type. On the other hand, several cystic changes including brachial cleft, thymic, bronchial, and esophageal cysts, may appear in the anterior mediastinal area and most of them are originated from the endoderm of the 3rd and 4th pharyngeal pouches. These cystic lesions are broadly classified according to their morphological features of the cyst walls and location. Interestingly, there are a few case reports of human thymomas arising in the wall of these thymic cysts [4, 13, 19], which are distinguished from cystic changes of thymomas.

The present paper describes the morphological features of multiple cysts containing neoplastic foci mimicking mixed type thymoma in the anterior mediastinal area of a dog suffering from acquired myasthenia gravis. The relationship between thymoma and multiple cysts is discussed.

An 11-year-old mongrel male dog started to show dysbasia, vomiting, and bad appetite on March 12, 2001, and was presented to a private animal hospital. Since the dog had typical clinical features of acquired myasthenia gravis, anticholinesterase drug, endrophonium chloride, was injected to confirm the diagnosis. By endrophonium chloride injection, apparent amelioration of dysbasia was observed. In addition, X-ray examination revealed the presence of mass lesion in the anterior mediastinal area and megaesophagus.

Needle biopsy of the anterior mediastinal mass revealed a large number of neutrophils, macrophages, and a few mesothelial cells and mast cells, but no neoplastic cells were detected. Diagnosis of the anterior mediastinal mass was not made at this time. Based on these findings the dog was clinically designed as acquired myasthenia gravis associated with some thymic lesions. The dog was successfully controlled for 134 days by anti-cholinesterase drug, pyridostigmine bromide, and predonisolone. The dog suddenly died of thermic stroke on July 25, 2001. Immediate necropsy at the hospital revealed diffuse pulmonary hemorrhage and fatty tissues in the anterior mediastinal area measured by 9.0 × 2.0 × 0.8 cm. Whole the anterior mediastinal tissue was fixed by 10% formalin for further pathological examinations.

The fixed tissue contained multiple cysts ranging from 2 to 10 mm in diameter. Some cysts were filled by yellowish pink fluid and some contained yellowish white solid nodules (Fig. 1). Paraffin sections of 2 to 4 μm thick were made and stained with hematoxylin and eosin (HE) and periodic acid Schiff (PAS) for routine histopathological examination. Immunohistochemical analysis was performed using Envision polymer reagent (Dako-Japan, Kyoto, Japan). As primary antibodies, rabbit antisera against human CD3 (1:40, Dako-Japan), keratin (wide range, prediluted, Dako-Japan), and mouse monoclonal antibody against cytokeratin (prediluted, Nichirei, Tokyo, Japan) were used. By routine histological examination, multiple cysts (Fig. 2), neoplastic tissues within cyst, and atrophic thymus were observed in submitted anterior mediastinal tissue. The cysts were lined by thin wall consisting of single to double layer of ciliated cuboidal and non-ciliated round epithelial cells (Fig. 3) and

the lumen was filled with eosinophilic colloidal fluid. Immunohistochemically, the ciliated epithelial cells were negative for keratin and cytokeratin, although non-ciliated epithelial cells were intensely positive for both antigens. Some largely extended cysts contained neoplastic tissue within the lumen and their cyst walls (Fig. 4). The neoplastic tissues consisted of mixed proliferation of large epithelial cells with abundant clear cytoplasm and large oval nuclei and lymphocytes (Fig. 5). The large epithelial cells sometimes formed glandular structures (Fig. 6) and sometimes showed single cell keratinization representing as eosinophilic spheroids (Fig. 7). By immunohistochemistry, proliferating epithelial cells were intensely positive for keratin and cytokeratin. In addition, infiltration of lymphocytes was abundant within the neoplastic foci, and more than half number of infiltrating lymphocytes was intensely positive for CD3 suggesting T cells (Fig. 8). Within the lumen of large cysts or around the neoplastic foci, there were abundant granulomatous areas consisting of accumulation of macrophages with yellowish brown pigments and cholesterol deposits.

The clinical features including megaesophagus and myasthenia gravis, were quite typical for the patients with thymoma [6, 9, 10, 12, 14, 17, 18, 22]. The present dog was clinically diagnosed as acquired myasthenia gravis before pathological examinations and was successfully controlled by anti-cholinesterase treatment. This fact indirectly suggests the present dog might be involved in autoimmune condition mediated by autoantibodies such as for acetylcholine, titin, or ryanodine receptors associated to thymic lesions [9, 18]. Since serological analysis to detect autoantibody was not performed in this dog, the real pathogenesis of myasthenia gravis remained unknown. However, these clinical signs were very informative for pathological diagnosis of thymomas or related thymic changes including thymic cysts.

The neoplastic lesions are considered as mixed type thymoma consisting of solid proliferation of thymic epithelial cells and lymphocytes. Glandular differentiation of large clear epithelial cells is unique morphological character of the present case, although similar changes have been also reported previously in canine thymomas [1, 15, 21]. Occasional keratinization of the epithelial cells might imitate Hassall's bodies that are specific feature of thymus and its

tumors. Moreover, most of the infiltrating lymphocytes showed intense reactivity for CD3, suggesting T cells. Thus, all these morphological features are consistent with those of canine thymomas [2, 3, 5, 7, 16]. Interestingly all neoplastic foci were formed within the lumen and wall of largely extended cysts. Cystic changes of thymomas were sometimes recognized in animals and humans and some were reported as cystic thymomas [8, 11]. Recently, Sugio *et al.* [19] reported a case of thymoma arising in the wall of the thymic cyst in a 77-year-old woman. In addition, several malignant thymic lesions arising from the thymic cysts have been reported in humans [4, 13]. In the present dog, there were multiple cysts varied in size in the anterior mediastinal area, together with atrophic thymus and some large cysts contained neoplastic lesions. It would be very difficult to decide whether present thymoma was arisen from the thymus with non-neoplastic cyst formation, or originated from the cyst walls. Since almost all neoplastic foci were recognized within the lumen and wall of large cysts, the present thymoma would be considered to arise in congenitally formed thymic cysts that related to brachial cleft tissues. The epithelial cells of these cysts may differentiate to thymic epithelial cells when they start to proliferate forming thymic tumors. In addition, some neoplastic epithelial cells may retain enough functional ability to mediate T cell-differentiation of lymphocytes, because most infiltrating lymphocytes in the neoplastic foci were immunopositive for CD3. On the other hand, human acquired thymic cysts are described as multiocular and consisted of variously thickened walls with severe inflammation [20]. Present thymic cysts represented as multiple, although inflammatory reactions were limited within the neoplastic foci. In addition, the cysts without neoplastic lesions were lined by thin wall consisting of single to double layer of ciliated and non-ciliated epithelial cells. Thus, except for the multiple formations, the present cysts contradict to multiocular thymic cysts in humans.

In conclusion, the present paper describes a unique morphology of canine thymoma probably arising from multiple thymic or brachial cleft cysts. The present case indicates that careful consideration should be needed for the diagnosis of such cystic changes in the mediastinal area, especially in dogs with acquired myasthenia gravis or other clinical signs associated to thymomas.

Fig. 1. The cut surface of large cysts (arrow heads), approximately 10 mm in diameter, are filled by yellowish fluid and contain white solid neoplastic tissues (arrows).

Fig. 2. Multiple cysts varied in size are filled by eosinophilic homogeneous fluid. HE. Bar = 1 mm.

Fig. 3. Ciliated cuboidal and non-ciliated round epithelial cells lining the cyst. HE. Bar=20  $\mu$ m

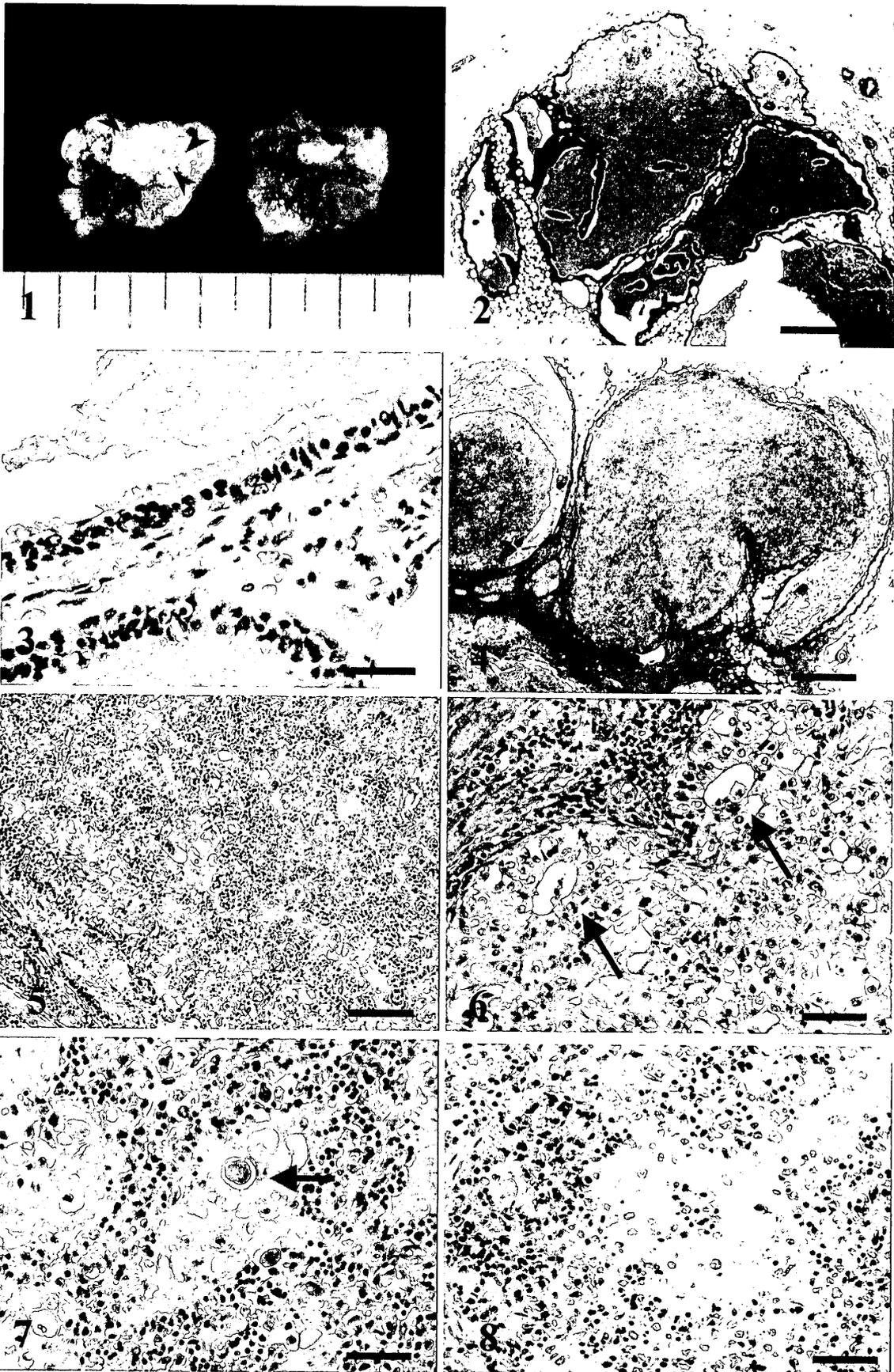
Fig. 4. Two large cysts containing neoplastic foci. HE. Bar=1 mm.

Fig. 5. Mixed population of large epithelial cells and lymphocytes within the neoplastic foci. HE. Bar=200  $\mu$ m.

Fig. 6. Glandular formation of proliferating large clear epithelial cells. HE. Bar = 20  $\mu$ m.

Fig. 7. Keratinization of proliferating large clear epithelial cell imitating Hassall's body. HE. Bar=20  $\mu$ m.

Fig. 8. Infiltration of CD-3 positive lymphocytes within the neoplastic tissue consisting of large epithelial cells. CD-3-immunostaining. Bar=20  $\mu$ m.



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*p53*ヘテロ欠損[*p53* (+/-)]および野生型[*p53* (+/+)]CBAマウスの肝発癌感受性を検討することを目的として、71匹の*p53*(+/-)および74匹の*p53*(+/+)CBAマウス(雄、6-12週齢)を用い、実験1ではflumequine(FL)を4,000ないし0 ppm混餌投与、あるいは実験開始時にdimethylnitrosamine(DMN)を5 mg/kg腹腔内単回投与、実験2ではdi(2-ethylhexyl)-phthalate (DEHP)を6,000ないし0 ppm混餌投与、実験3ではphenolphthalein(PhP)を12,000, 6,000ないし0 ppm混餌投与した。26週間の処置後、すべての生存動物を屠殺した。FL群の肝細胞小増殖巣の発生頻度は*p53*(+/+)マウスにおいて、その発生個数は*p53*(+/-)、*p53*(+/+)マウスにおいて対照群に比し有意に増加した。DMN群の肝細胞小増殖巣の発生頻度と発生個数は、対照群に比し軽度増加した。また、FL、DMN群ともに肝細胞小増殖巣の発生頻度、発生個数およびPCNA陽性細胞数比は*p53*(+/-)と*p53*(+/+)マウスとの間で有意差は認められなかった。DEHP、PhP群ともに肝細胞小増殖巣の発生頻度や発生個数は対照群に比し有意差は認められなかった。以上の成績より、CBAマウスにおける*p53*遺伝子の片側欠損は化学物質による肝発癌誘導を増強しないことが示唆された。

21カ月齢サラブレッド種馬にみられた腸管スピロヘータ症(短報)——芝原友幸<sup>1)</sup>・桑野睦敏<sup>2)</sup>・上野孝範<sup>2)</sup>・安斉了<sup>3)</sup>・桑本康<sup>3)</sup>・佐藤宏昭<sup>4)</sup>・前田泰治<sup>1,5)</sup>・石川義春<sup>1)</sup>・門田耕一<sup>1)</sup>(<sup>1)</sup>独立行政法人農業技術研究機構動物衛生研究所北海道支所、<sup>2)</sup>日本中央競馬会競走馬総合研究所、<sup>3)</sup>同・栃木支所、<sup>4)</sup>同・日高育成牧場、<sup>5)</sup>北海道根室家畜保健衛生所).....

633-636

7カ月間、持続的な下痢と発育遅滞がみられた21カ月齢サラブレッド種仔馬が病性鑑定に供された。剖検時、盲腸と結腸粘膜は瀰漫性に粗糙で充血していた。病理組織学的に、粘膜と粘膜下組織は水腫性で、多数のリンパ球や大食細胞の浸潤がみられた。同時に、*Brachyspira*抗原をもち形態的に3種のスピロヘータが盲腸と結腸病変部上皮の粘液層と陰窩に多数認められた。それらは、しばしば変性上皮細胞の細胞質内ならびに細胞間と粘膜固有層、特に血管周囲に認められた。これら侵入性腸管スピロヘータは馬に大腸炎ならびに下痢を起こす病原体のひとつである可能性が示唆された。

後天性重症筋無力症罹患犬にみられた胸腺腫および多発性胸腺嚢胞(短報)——内田和幸<sup>1)</sup>・粟村雄一<sup>2)</sup>・中村智子<sup>1)</sup>・山口良二<sup>1)</sup>・立山晋<sup>1)</sup>(<sup>1)</sup>宮崎大学農学部家畜病理学教室、<sup>2)</sup>戸塚動物病院).....

637-640

11歳雑種犬の前縦隔部に認められた嚢胞性病変を検索した。本例は虚脱と巨大食道症に起因する嘔吐を示し、X線により前縦隔部に直径約35 mmの球形腫瘤病変が確認された。臨床試験により本例は後天性重症筋無力症と診断され、抗コリンエステラーゼ薬治療で約4ヶ月間良好に維持された。本例は熱性ショックにより死亡し剖検された。肉眼的に前縦隔部には、黄色調液体を貯留する嚢胞と白色結節を含む脂肪組織が認められた。組織学的に同組織には、多発性嚢胞、腫瘍組織、および萎縮した胸腺が含まれていた。嚢胞は長立方形の線毛上皮と円形非線毛上皮からなる薄い嚢胞壁から構成され、内部に好酸性コロイドを有していた。一部の拡張した嚢胞内及び嚢胞壁には腫瘍組織がみとめられ、この腫瘍組織は、淡明な細胞質と大型円形核を有する大型上皮細胞とリンパ球から構成されていた。免疫組織化学的に上皮細胞はケラチンおよびサイトケラチン強陽性で、浸潤していたリンパ球の半数以上がCD3陽性でありT細胞と考えられた。以上の検索結果より腫瘍性病変は胸腺腫、多発性嚢胞は胸腺嚢胞あるいは遺残鰓弓性嚢胞と考えられた。

副甲状腺ホルモンによる若齢ラットの骨間質細胞からのインターロイキン6遺伝子発現の誘導(短報)——千葉修一<sup>1,2)</sup>・海野正子<sup>3)</sup>・Neer, R. M.<sup>1)</sup>・岡田幸助<sup>2)</sup>・Segre, G. V.・Lee, K.<sup>2)</sup>(<sup>1)</sup>ハーバード大学マサチューセッツ総合病院内分泌科、<sup>2)</sup>岩手大学農学部家畜病理学教室、<sup>3)</sup>中外製薬(株)富士御殿場研究所).....

641-644

副甲状腺ホルモン(PTH)が骨局所においてインターロイキン6(IL-6)の発現を制御するかを検証するため、4週齢のラットにPTH 1-84 225 µg/kgを単回皮下投与し、*in situ*ハ