Spinal Oligodendroglioma with Diffuse Arachnoidal Dissemination in a Japanese Black Heifer

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ABSTRACT. A gelatinous focus with cystic spaces, was found in the posterior funiculus of the 2nd to 3rd lumbar levels of the spinal cord of a Japanese Black heifer, 2 years old, with clinical signs of severe dysstasia. Histopathological examination revealed that the spinal lesion consisted of multifocal and diffuse proliferation of round cells with abundant vacuolar cytoplasm and hyperchromatic nuclei. In the lesions there was a number of cystic spaces containing aggregates of small round cells. The neoplastic foci showed a honeycomb structure divided by thin blood vessels, representing typical lesions of oligodendroglioma. Diffuse and multifocal proliferation of these round cells were also recognized in the subarachnoidal space in the sacral spinal cord. Immunohistochemically, the proliferating round cells were negative for glial fibrillary acidic protein. Based on these morphological features, the case was diagnosed as lumbar spinal oligodendroglioma with diffuse arachnoidal dissemination.—KEY WORDS: bovine, oligodendroglioma, spinal cord.
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Oligodendroglioma is a common brain tumor of animals, especially dogs [13], but only one bovine case has been reported [1]. Although both animal and human oligodendrogliomas are known to occur predominantly in the white matter of the cerebral hemispheres [9, 12, 13], spinal oligodendrogliomas are also recognized occasionally in children, and their clinical and pathological natures have been well demonstrated in several human cases [2]. In contrast, spinal cord tumors including oligodendrogliomas [5, 15] are rare in animals, except for extra-medullar tumors such as spinal nephroblastomas in young dogs [13], and bovine spinal gliomas are considered to be extremely unusual [1].

The present paper describes the morphological and immunohistochemical features of a spinal oligodendroglioma in a 2-year-old Japanese Black heifer and discusses the similarity between human and bovine cases.

A 2-year-old Japanese Black heifer suddenly developed hind limbs paralysis and dysstasia. The animal was treated by clysis with Ringer's solution, but the clinical signs did not improve. The animal was euthanatized by electric shock 7 days after clinical onset at the owner's request, and necropsied immediately. In the brain, there were no significant lesions, but congestion and yellowish discoloration was recognized in the posterior area of the 2nd to 3rd levels of the lumbar spinal cord. The cut surface in this area revealed a pale gelatinous focus, approximately 1 cm in diameter, with multiple cystic spaces filled with clear fluid, located mainly in the posterior funiculus (Fig. 1). The gelatinous focus was attached to the dura mater, and the subarachnoidal space was replaced by the gelatinous material. In the subarachnoidal space of the lower lumbo-sacral spinal cord, a small amount of fibrinous material, pale to tan in color, was distributed diffusely and widely.

Tissue samples were fixed in 10% formalin and routinely processed. Paraffin sections were stained with hematoxylin and eosin (HE), and some selected sections of the spinal cord were also stained with Luxol fast blue (LFB), Masson's trichrome, Watanabe's silver impregnation, and alcian blue (pH 2.5). Histopathological examination revealed that the lumbar spinal lesion consisted of multifocal and diffuse proliferation of neoplastic cells with cystic spaces (Fig. 2). The tumor mass was located mainly in the white matter, and the posterior funiculus were almost totally replaced by the proliferating tumor cells and cystic places. The neoplastic foci showed a honeycomb structure with solid proliferation of round cells, representing the typical appearance of oligodendroglioma (Fig. 3). Most neoplastic cells were round in shape with abundant clear cytoplasm and hyperchromatic central nuclei. The presence of cytoplasmic processes was not confirmed. In the tumor there were a number of blood vessels with thin vascular walls, while the endothelial cells did not show apparent hyperplastic or proliferative changes. There were also a few of well differentiated astrocytes with well defined eosinophilic cytoplasmic processes in the neoplastic foci. In contrast, at the periphery of the neoplastic foci, diffuse proliferation of these astrocytes was prominent. Within the cystic spaces, there were aggregates of small number of small round cells mimicking lymphocytes, with small hyperchromatic round nuclei and scanty cytoplasm. Mitoses of the neoplastic cells were extremely rare. The neoplastic cells invaded the leptomeninges, and the subarachnoidal space was filled by a proliferation of these cells. Diffuse and multifocal proliferation of the round cells and lymphocyte-like small round cells, were also seen in the subarachnoidal space in the 2nd and 3rd levels of the sacral spinal cord (Fig. 4). In this area, some foci consisted of aggregates of neoplastic cells, and some areas showed an organoid structure made up of tumor cells, a few astrocytes and their cytoplasmic processes (Fig. 5). Besides the spinal cord there was no evidences for metastasis of the spinal cord tumor.

Immunohistochemistry was performed using the Envision polymer method (Dako Japan Co. Ltd., Kyoto, Japan). The

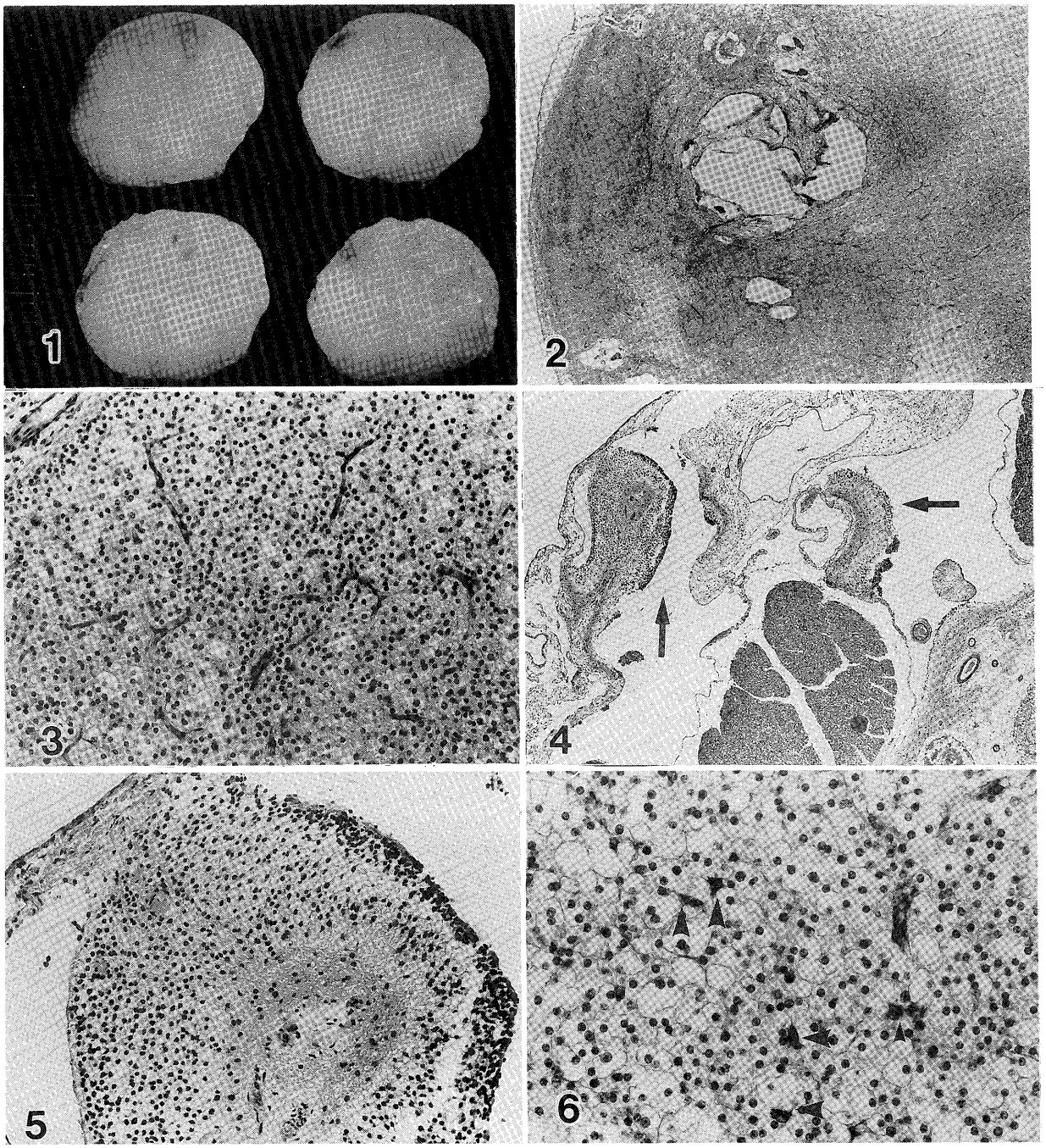

Fig. 1. A gelatinous foci with cystic spaces mainly in the posterior funiculus of the lumbar spinal cords (L2 to L3).
Fig. 2. Diffuse proliferation of neoplastic cells with forming many cystic spaces in the posterior funiculus of the lumbar spinal cord (L2). HE. × 10.
Fig. 3. A honeycomb structure with accumulation of round neoplastic cells with a proliferation of blood vessels. HE. × 200.
Fig. 4. Multiple foci (arrows) consisting of a proliferation of neoplastic cells in the subarachnoidal space of the sacral spinal cord (S3). HE. × 50.
Fig. 5. High power magnification of Fig. 4. Neoplastic focus showing organoid structures. HE. × 100.
Fig. 6. A few number of GFAP-positive astrocytes (arrow heads) in the neoplastic foci of the lumbar spinal cord. The round tumor cells with abundant clear cytoplasm are negative for GFAP. GFAP-immunostain. × 300.

following antibodies were used as primary antibodies; rabbit antisera against human glial fibrillary acidic protein (GFAP, Prediluted, Dako), bovine galactocerebroside (GC, 1:200, Chemicon, Temecula, CA, U.S.A.), bovine S-100 (1:400, Dako), myelin basic protein (MBP, prediluted, Zymed Laboratories, South San Francisco, CA, U.S.A.) and neuron-specific enolase (NSE, 1:200, Dako), and mouse monoclonal antibodies against human neurofilament (NF, 1:20, Dako),

vimentin (1:40, Dako), synaptophysin (1:20, Dako), and proliferating cell nuclear antigen (PCNA, prediluted, Dako). To visualize microglia, sections were stained with biotinylated lectin *Ricinus communis agglutinin-1* (RCA-1, 1:400, EY Laboratories, San Mateo, CA, U.S.A.). The lectin staining was done using the avidin-biotin peroxidase complex method (Vector Laboratories, Burlingame, CA, U.S.A.). Immunohistochemically, neoplastic round cells with abundant clear cytoplasm and small lymphocyte-like cells were completely negative for GFAP. Only a few of GFAP-positive astrocytes were recognized within the neoplastic foci (Fig. 6), and many GFAP-positive astrocytes were distributed at the periphery. Immunostaining for MBP and GC labeled myelin sheaths and intact oligodendrocytes intensely, and intact and reactive astrocytes also showed moderate immunoreactivity for GC. Neoplastic round cells were moderately positive for GC and negative for MBP. The neoplastic cells were negative for other antibodies, including NF, synaptophysin, vimentin, S-100, NSE, and lectin RCA-1. The number of tumor cells with PCNA-positive nuclei was very small, approximately 3 to 5 cells under high-power magnification (10 × 40). A similar number of PCNA-positive astrocytes were detected at the periphery of the neoplastic foci. The results of PCNA staining indicate a poor growth activity of the neoplastic cells.

Typically oligodendroglioma forms soft gelatinous mass, occasionally containing cystic spaces and leptomeningial or ventricular extension is quite common [2, 8, 14]. Histopathologically, this tumor is characterized by a honeycomb appearance formed by accumulation of tumor cells with artifactualy swollen clear cytoplasm and central nuclei [9, 12, 13]. Several immunohistochemical markers such as MBP, GC, and Leu 7, have been applied for diagnosis of human oligodendrogliomas [10, 12], although the findings have varied from case to case. These previous studies indicated there were no specific markers for the diagnosis of oligodendrogliomas. Moreover, the co-presence of GFAP-positive cells in oligodendrogliomas [4, 6] becomes problematic when trying to differentiate pure oligodendroglioma from mixed glioma (oligo-astroglioma).

The present spinal tumor had the histological characteristics of typical oligodendroglioma reported in humans and several animal species [9, 12, 13]. The tumor showed a honeycomb appearance formed by accumulation of round cells with vacuolated cytoplasm, and these neoplastic cells were negative for GFAP. Cystic spaces containing small round neoplastic cells, were prominent in this tumor. In both human and animal oligodendrogliomas, similar cystic changes have been commonly described, and are considered to occur by mucinous degeneration of the tumor [9, 12, 13, 15]. Based on these morphological features, the present case was diagnosed as oligodendroglioma. Recently, it has been reported that some brain tumors including astrocytoma, ependymoma, or central neuroma, can occasionally exhibit honeycomb-like structures [3, 7]. However, the negative immunoreactivity of the tumor for GFAP, NSE, NF and synaptophysin may support our diagnosis. A few GFAP-positive astrocytes were present in the neoplastic foci, and diffuse proliferation of GFAP-positive astrocytes was recognized at the tumor periphery. These GFAP-positive astrocytes were considered to be reactive astrocytes. The presence of a sub-population of neoplastic cells with astrocytic differentiation known as minigemistocytes or gliofibrillary oligodendrocytes has been demonstrated in many human oligodendrocytic tumors [4, 11]. However, we were unable to confirm the neoplastic nature of the GFAP-positive cells in the present case. Since histopathological differentiation between pure oligodendroglioma and mixed glioma (oligo-astroglioma) may be quite difficult, mixed glioma could still be considered in the differential diagnosis of this tumor. Anaplastic (malignant) oligodendrogliomas are characterized by anaplastic morphology and prominent mitotic activity of tumor cells, vascular endothelial proliferation, and abundant necrosis sometimes with calcium deposits [9, 12, 13]. In spite of the wide arachnoidal dissemination, the tumor cells showed the typical morphology of oligodendroglioma with low growth activity, suggested by the extreme rarity of mitotic figures and PNCA immunoreactivity. Moreover, endothelial proliferation, necrosis, and calcification were not prominent in the present tumor. Thus, these morphological features are thought to be quite different from those of anaplastic oligodendroglioma.

Although there are few reports of spinal oligodendrogliomas in animals [5, 15], the spinal white matter may be affected. Even in humans spinal oligodendrogliomas are described as rare, and most cases have arisen in children [2, 8, 14]. Radiation therapy is considered to be effective for this tumor, but many cases have a poor clinical prognosis. Nam *et al.* [8] mentioned that the poor prognosis of spinal oligodendroglioma might be due to its biological nature frequently showing wide extension or dissemination. In their review, Fortuna *et al.* [2] cited several articles in which intracranial or spinal meningeal extension of oligodendroglioma known as "oligodendrogliomatosis", was reported as frequent, seven of 10 necropsied cases showing pathological evidence for this event. In oligodendrogliomas of animals, similar biological features have been emphasized [13], although such findings might be based mainly on canine cerebral oligodendrogliomas, including anaplastic oligodendrogliomas. In the present case, diffuse arachnoidal dissemination of the tumor cells was found in the subarachnoidal space from the 2nd lumbar to 3rd sacral levels of spinal cord, in spite of the well differentiated morphological features and low mitotic activity. Since diffuse invasion of neoplastic cells in the subarachnoidal space was recognized at the primary site, tumor seeding might have occurred easily and widely to the lower part of the spinal cords through the cerebrospinal fluid. These findings indicate that bovine spinal oligodendroglioma has similar biological characteristics to that of the human form. It is difficult to appraise the clinical features of the present

tumor, although the wide subarachnoidal dissemination indicates a poor prognosis, as in human cases.

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日本各地より分離した*Eimeria tenella*株間でのDNA多型をPCRで増幅した1.1 kb小サブユニットリボゾームRNAを用いて検討した．遺伝子の変化はrandom amplification of polymorphic DNA法で調べた．その結果，DNAフィンガープリントの型は2つに分けられたことから，日本の*Eimeria tenella*には少なくとも2つのDNA多型が存在することが示唆された．

本邦東北地方北西部に生息する野生食肉目の蠕虫相，特に未記録種*Mesocestoides paucitesticulus*および*Brachylaima tokudai*(短報)——佐藤　宏・井濱　康・稲葉孝志・八木澤　誠・神谷晴夫（弘前大学医学部寄生虫学教室） **1339-1342**

1998－1999年の冬季に，本邦東北地方北西部で捕獲されたホンドテン38頭，ホンドタヌキ14頭，ホンドキツネ2頭について寄生虫学的検討を行った．これら野生動物から線虫11種，吸虫10種，条虫3種，鉤頭虫1種が回収され，本州あるいはこの地方から未報告の蠕虫5種が確認された．特に，テンからの*Mesocestoides paucitesticulus*，タヌキからの*Brachylaima tokudai*は新宿主記録である．

病　理　学：

黒毛和種牛における瀰漫性クモ膜内播種を伴う脊髄稀突起膠細胞腫の一例(短報)——内田和幸・村中美樹・村上隆之[1]・山口良二・立山　晉(宮崎大学農学部獣医学科家畜病理学教室，[1]家畜解剖学教室) **1323-1326**

重度の運動失調を示した2歳，雌，黒毛和種牛の第2～3腰椎部脊髄に嚢胞形成を伴うゼラチン状病巣が認められた．病理組織検索の結果同病巣では，豊富な空胞状の細胞質とクロマチンに富む核を有する円形細胞の多巣状性および瀰漫性増殖と小型円形細胞の集簇を含む多数の空胞病変が確認された．本病巣は，稀突起膠細胞腫に特徴的な蜂巣状構造を示し，薄い血管により区画されていた．また仙椎部脊髄の検索では，クモ膜下腔において瀰漫性ないし多巣状性の円形細胞増殖部が確認された．免疫組織化学的に，増殖性円形細胞はグリア線維性酸性蛋白（GFAP）に陰性を示した．以上の検索結果より，本例は瀰漫性クモ膜播種を伴う腰髄稀突起膠細胞腫と診断された．

日本白色種ウサギにおける自然発生リンパ腫(短報)——渋谷一元・田島正典・金井一享・伊原三重子・布谷鉄夫((財)日本生物科学研究所) **1327-1329**

4ヶ月齢の日本白色種雌ウサギの1匹にリンパ腫がみられた．肉眼的に，腸間膜リンパ節および空腸パイエル氏板の著明な腫大，脾腫，気管気管支リンパ節，副腎および卵巣の腫大が観察された．組織学的に，リンパ球様腫瘍細胞は頻繁な細胞分裂像および特徴的な星空像を示しながらびまん性に増殖していた．腫瘍細胞の好塩基性細胞質には数個の脂肪滴が含まれていた．腸間膜リンパ節，空腸パイエル氏板および気管気管支リンパ節ではその殆どが組織が腫瘍組織に置き換わっていた．胃，小腸，肝臓，脾臓，卵巣および副腎では腫瘍細胞が高度に浸潤していた．これらの結果は，今回のリンパ腫が胃腸管のリンパ組織に由来したことを示唆している．

末梢神経線維を包含したイヌの末梢神経鞘腫の1例(短報)——澤本　修[1,2]・山手丈至[1]・桑村　充[1]・萩原里香[2]・栗栖和信[2]([1]大阪府立大学大学院農学生命科学研究科獣医病理学講座，[2](株)大塚製薬工場鳴門研究所) **1335-1338**

7歳齢の雄イヌの右頬部皮下と口腔粘膜下に末梢神経鞘腫が認められた．腫瘍細胞は紡錘形で束状に配列し，異型性に乏しく，核分裂像もほとんど認められなかった．電顕的に神経鞘に特徴的な基底膜を有していた．腫瘍内には神経線維が観察された．免疫組織化学的に腫瘍細胞はビメンチン，glial fibrillary acidic protein，S-100蛋白及びneuron specific enolaseに，腫瘍内の神経線維はmyelin basic protein及びneurofilament proteinにも陽性を示した．すでに報告されているイヌの血管周皮腫と比較し，鑑別診断上の考察を加えた．