A porcine lymphoma-derived cell line co-expressing IgM, IgG and IgA

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ABSTRACT. A cell line (PL38PB) was established from blood samples of a 6-month-old pig that was diagnosed with lymphoma with CD5 expression. Histopathological examination revealed neoplastic lesions in the spleen, liver and lymph nodes. Tumor cells were immunohistochemically positive for CD20 and immunoglobulin heavy chains (μ, γ and α). Membranous CD5 and cytoplasmic Immunoglobulin M (IgM), Immunoglobulin G (IgG) and Immunoglobulin A (IgA) were detected in PL38PB cells by flow cytometry. In addition, the cytoplasm of PL38PB cells were positive for IgM, IgG and IgA by immunofluorescent. However, no Ig secretion was detected in culture supernatant by Ouchterlony gel diffusion method. Results suggest that PL38PB cells express three Ig isotypes that are produced but not secreted.

KEYWORDS: CD5, cell line, immunoglobulin, lymphoma, swine

Lymphoma is the most frequently reported neoplasm in swine, according to surveys in slaughterhouses. Unlike in cattle, swine lymphoid neoplasms are relatively similar in histology to their counterparts in humans [22]. For example, signet ring cell lymphoma in humans is a variant of follicular lymphoma characterized by eosinophilic Russell body-type globules representing intracytoplasmic retention of immunoglobulin M (IgM) [2], and very similar cases have been reported in pigs [14, 21]. Other Ig-producing tumors, such as lymphoplasmacytic lymphoma [13, 20], diffuse centroblastic lymphoma, intestinal large B cell lymphoma [22], immunoblastic lymphoma and plasmacytoma [13] have been recorded in swine.

The advent of leukemia-lymphoma cell lines has contributed to a better understanding of the pathophysiology of hematopoietic tumors [3]. In human leukemia-lymphoma cell lines, the originating neoplasms are categorized into distinct histological types [6, 17]. Hence, we can compare features of the cell lines and the neoplasms from which they were derived. In contrast, in swine no histological diagnosis had been made in nearly all of the original lymphoid neoplasms from which immortal cell lines have been derived [15], although cell lines have been established from a case of immunoblastic lymphoma in a hog [11] and a case of centroblastic lymphoma in a market weight pig [24]. Here, we report a case of swine lymphoma and a cell line established therefrom.

A 6-month-old crossbred castrated male pig was brought to an abattoir. At necropsy, the spleen and liver were severely enlarged, and some superficial, thoracic and abdominal lymph nodes were also enlarged. The rib bone marrow was red in color. For histopathological analysis, all enlarged tissues and born marrow were fixed in 10% buffered formalin, embedded in paraffin, sectioned at a thickness of 4 µm, and stained with hematoxylin and eosin (HE). Subsequently, some sections were labeled by the streptavidin-biotin-peroxidase complex (SAB) method with a SAB kit (Nichirei, Tokyo, Japan). The primary antibodies used are presented in Table 1. There was no cross reaction among antisera to heavy chains.

After calculating the white blood cell (WBC) count of the residual blood in the mesenteric blood vessels, a blood smear was made and stained with May-Grünewald-Giemsa (Merck, Darmstadt, Germany). The blood was layered on Ficoll-Hypaque (density: 1.077, Amersham Pharmacia Biotech, Piscataway, NJ, USA), centrifuged, and used for culture. Cells were grown in RPMI 1640 (Gibco, Grand Island, NY, USA) containing 10% fetal bovine serum (FBS), L-glutamine (2 mM), 2-mercaptoethanol (5 × 10⁻⁵ M), 0.011% sodium pyruvate, 0.1% spectinomycin, and 0.0075% NaHCO₃. The number of viable cells was adjusted to 2 × 10⁶
cells/ml, and static culture was started at 37°C in 5% CO₂. Constant growth was observed in the cells from the 150th passage, and the established cell line named PL38PB was examined morphologically and immunohistochemically.

For electron microscopy, 1.0 × 10⁸ PL38PB cells were collected, fixed in 2.5% glutaraldehyde at 4°C for 1 hr, and washed three times with phosphate-buffered saline (PBS). The samples were solidified with 4% agar, cut into 1 mm³ cubes, post-fixed with 1% osmium tetroxide, dehydrated with alcohol, replaced with QY-1, and embedded in epoxy resin. Ultrathin sections were double-stained with uranyl acetate and lead citrate, and observed under a transmission electron microscope (JEM 1210, JEOL, Tokyo, Japan).

Dual fluorescent immunostaining was performed using antibodies listed in Table 1 as primary antibodies in various combinations. A Zenon Tricolor Alexa Fluor labeling kit (Invitrogen Carlsbad, CA, USA) and Phicoerythrin-labeled rabbit anti-goat IgG (Rockland, Gilbertsville, PA, USA) were utilized as secondary antibodies. Flow cytometry was performed to analyze the ratio of cells positive for Igs and cell surface proteins among PL38PB cells. As primary antibodies, mouse anti-swine CD2, CD3, CD4, CD5, CD6, CD8α, CD14, CD21 and CD45RA (VMRD, Pullman, WA, USA) were used. The other antibodies are given in Table 1. Fluorescein isothiocyanate (FITC)-labeled rabbit anti-goat IgG (Cappel, Durham, NC, USA) and FITC-labeled goat anti-mouse IgG (Cappel) were used as secondary antibodies. Analysis was carried out by Epics XL/XL-MCL (Beckman Coulter, Fullerton, CA, USA) as the measuring instrument.

The presence of Ig in culture supernatants was investigated using the Ouchterlony method [23], and chromosomal karyotyping was also performed.

The WBC count in residual blood was 110,000/µl. Most white blood cells were larger in size than normal lymphocytes (Fig. 1).

**Table 1.** Primary antibodies used for immunohistochemistry, fluorescent immunostaining and flow cytometry

| Antibody | Clone | Type | Label | Dilution | Manufacturer |
|----------|-------|------|-------|----------|--------------|
| **Immunohistochemistry** | | | | | |
| Swine IgM (μ chain specific) | RpAb | 1:1,600 | Bethyl Laboratories, Montgomery, TX, USA |
| Swine IgG (Fc specific) | GpAb | 1:1,600 | Bethyl |
| Swine IgA (α chain specific) | RpAb | 1:1,600 | Bethyl |
| Human κ light chain | RpAb | 1:1,600 | Bethyl |
| Human λ light chain | RpAb | 1:1,600 | Bethyl |
| Human CD20 | RpAb | PD | Spring Bioscience, Pleasanton, CA, USA |
| Human CD5 | RpAb | PD | Lab Vision, Fremont, CA, USA |
| Human CD3 | RpAb | 1:50 | Dako A/S, Glostrup, Denmark |
| **Fluorescent immunostaining** | | | | | |
| Swine IgM * | GpAb | 1:50 | Bethyl |
| Swine IgG * | GpAb | 1:50 | Bethyl |
| Swine IgG | RpAb | 1:100 | Cappel |
| Swine IgA K61 1B4 | mAb | 1:10 | Bio-Rad Laboratories, Hercules, CA, USA |
| **Flow cytometry** | | | | | |
| Swine IgM | GpAb | 1:10 | Bethyl |
| Swine IgG | GpAb | 1:10 | Bethyl |
| Swine IgA | GpAb | 1:10 | Bethyl |
| Swine CD2 MSA4 | mAb | 1:10 | VMRD, Pullman, WA, USA |
| Swine CD3 8E6 | mAb | 1:10 | VMRD |
| Swine CD4 72-12-4 | mAb | 1:10 | VMRD |
| Swine CD5 PG114A | mAb | 1:10 | VMRD |
| Swine CD6 PG90A | mAb | 1:10 | VMRD |
| Swine CD8a 76-2-11 | mAb | 1:10 | VMRD |
| Swine CD14 CAM36A | mAb | 1:10 | VMRD |
| Swine CD21 NZ1 | mAb | 1:10 | VMRD |
| Human BLA36 A27.42 | mAb | 1:10 | Novocastra, Newcastle, UK |
| Swine CD44 PORC24A | mAb | 1:10 | VMRD |
| Swine CD45RA MIL13 | mAb | 1:10 | Bio-Rad (Formerly AbD Serotec) Hercules, CA, USA |
| Swine CD79b AT107-2 | mAb | 1:10 | Gene Tex, CA Irvine, USA |

RpAb, rabbit polyclonal antibody; GpAb, goat polyclonal antibody; mAb, mouse monoclonal antibody; FITC, fluorescein isothiocyanate; PD, prediluted; MH, microwave heating. *Also used in flow cytometry.

Fig. 1. Cytology. Most white blood cells are larger in size than normal lymphocytes. May-Grünwald-Giemsa. Bar=50 μm.
Chromatin was less condensed than that of small lymphocytes. The cytoplasm was moderate to abundant.

Histologically, neoplastic proliferation was observed in the macroscopically visible lesions, and also in the rib bone marrow. The neoplastic tissues were composed mostly of medium-sized to large lymphoid or plasmacytoid cells with round, oval or slightly irregular nuclei and slightly to moderately condensed chromatin. The nucleoli were small- to medium-sized or rarely large, and the cytoplasm was scant to abundant (Fig. 2A). Immunohistochemically, CD5-positive lymphoma cells were scarcely found in most areas because of poor or prolonged fixation, but positive cells were present just beneath the capsule (Fig. 2B). Tumor cells were immunohistochemically positive for CD20 and a few μ-, γ- or α-positive lymphoma cells were detected (Fig. 2C). Lymphoma cells frequently expressed λ light chain. A few κ-positive cells resembling normal plasma cells were detected.

The doubling time of PL38PB cells was approximately 30 hr. May-Grünwald-Giemsa staining revealed that PL38PB cells had round or sometimes irregular nuclei and basophilic cytoplasm (Fig. 3). The cells were positive for λ light chain, but not for κ chain (Fig. 4). Electron microscopy revealed that the cells had round to horseshoe-shaped nuclei, abundant rough endoplasmic reticulum (rER), and microvilli on the cell surface (Fig. 5).

Double staining with fluorescent antibodies showed that PL38PB cells were dually positive for IgM and IgG (Fig. 6), IgM and IgA, and IgG and IgA. In flow cytometry analysis, PL38PB cells expressed IgG (84.4%), IgA (68.3%) and IgM (62.1%). Markers such as CD44 (76.6%), CD5 (63.5%), BLA36 (25.3%) and CD79b (18.4%) were also expressed (Fig. 7). The other markers were negative. Using the Ouchterlony method, positive precipitates lines of IgM, IgG and IgA were observed in normal swine sera, but not in culture supernatants. PL38PB cells had a chromosome number of 13 to 38 with a mode of 23, and 3% of the cells showed the normal number of pig chromosomes (2n=38).

In the WHO classification for human lymphoid neoplasms, chronic lymphocytic leukemia (CLL) is characterized by proliferation of small lymphoid cells with clumped chromatin and expression of CD5. Similar morphological and immunophenotypic features are observed in small lymphocytic lymphoma (SLL), but patients show clinical manifestations of lymphoma and no elevation of WBC count [18]. Plasmacytic differentiation can be seen in some of these neoplasms, but additional information is not available in the WHO classification. In the Kiel classification, which is the basis of the WHO classification, such cases are classified into...
lymphoplasmacytoid lymphoma, and some cases show a large number of immunoblastoid or centroblastoid cells, but the most predominant are small lymphoid cells [5, 16]. In the present case, CD5 and Ig were expressed, but the lymphoma cells were medium-to-large-sized. Hence a diagnosis of lymphoma with CD5 expression was made. Considering large cell transformation in human and bovine lymphoplasmacytoid lymphoma [9, 16], the possibility that the present case was in an advanced stage of lymphoplasmacytoid lymphoma remains.

In the case described here, a cell line, PL38PB, was established from the blood. The cells were positive for CD5, IgM, IgG and IgA by immunofluorescence and flow cytometry. Additionally, well-developed rER was observed ultrastructurally. Such results were consistent with immunohistochemical features in the original tumor. No Ig secretion was noted in culture supernatants. These
imply that cultured cells were capable of synthesizing but not secreting Igs, and the current case may be a non-secretory type of lymphoma with plasmacytic differentiation [4]. The paucity of lymphoma cells expressing CD5 or Ig heavy chains in tissue sections were due to poor or prolonged fixation of neoplastic tissues, and it was not easy to judge whether positive cells were neoplastic or not.

In general, neoplastic cells produce a single class of Ig in lymphoid neoplasms. In a case of swine follicular center cell lymphoma with immunoblastic transformation, immunoblastoid cells with only IgM or both IgM and IgG were observed, and the two kinds of Ig were considered to be temporarily expressed during class switching [12]. On the other hand, the simultaneous presence of cytoplasmic IgG and IgA was demonstrated in a swine plasmacytoma, and was considered to be associated with unusual RNA splicing or an abnormal event in allelic exclusion [13]. In humans, biphenotypic myeloma with dual expression of κ and λ chains has been reported [10], and triclonal gammopathy has been recorded in patients with myeloma [1, 19]. These phenomena were thought to be associated with RNA splicing, allelic exclusion or class switch recombination [7, 8, 26]. Two or more class switch DNA recombination patterns of Ig heavy chain or Ig light chain could be observed in single B cells from healthy human donors [25]. In the present case, at least two classes of Ig were demonstrated in single cultured cells.

POTENTIAL CONFLICTS OF INTEREST. The authors have nothing to disclose.

ACKNOWLEDGMENTS. The authors would like to thank Dr. Akinori Shimada (Laboratory of Pathology, School of Life and Environmental Science, Azabu University) for engaging useful discussions with us, as well as Ms. Miwako Kamiie (Laboratory of Veterinary Pathology, School of Veterinary Medicine, Azabu University) for her help with the experiments.
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