Ultrastructural Observation on Cultured Cells from Swine Immunoblastic Sarcoma

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B-immunoblasts are precursor cells of plasma cells and contain cytoplasmic immunoglobulin (CIg). Immunoblastic sarcoma, a malignant tumour of B-immunoblast, has been reported in a pig and was characterized by CIgG and well-developed rough surfaced endoplasmic reticulum (RER) [6]. The simultaneous presence of two immunoglobulins in single immunoblastic sarcoma cells could be explained by the heavy chain class switch [5].

Intracisternal A particles are present in a variety of normal and neoplastic mouse tissues and have been found in tumours of the gerbil, rat, guinea pig and man [12]. Such particles have not been seen in pigs, although C-type particles were observed in myeloid leukaemic pigs [2, 7] and various cell lines including a cell line from a porcine lymphoma [10].

In this paper, we report a morphological observation on cultured cells, which were derived from a swine immunoblastic sarcoma and had intracisternal A particles within slightly to relatively well-developed RER. The process to establish cell lines will be described in a separate publication.

A 6-month-old crossbred hog was brought to an abattoir with slight emaciation. At necropsy, the mandibular, axillary, superficial inguinal and caudal mesenteric lymph nodes were enlarged and some neoplastic masses were present in the subcutaneous tissues or skeletal muscles. There were multiple nodules in the lungs and several nodules in the heart and diaphragm.

After necropsy, tissues were fixed in 10% formalin, processed and embedded in paraffin wax, and sections were stained with haematoxylin and eosin (HE), Giemsa and periodic acid-Schiff (PAS). The peroxidase-antiperoxidase (PAP) immunoperoxidase method for gamma, mu, alpha and light chains was performed as described elsewhere [11].

Two types of cultured cells were obtained from the neoplastic tissue of the involved pig. In one type the cells were attached to the glass wall (Cell 1) and the other showed floating cells (Cell 2). Afterwards larger cells (Cell 3) were obtained from the culture of Cells 2, although Cell 1 died out. For electron microscopy, these cells were fixed with a mixture solution of 4% formaldehyde and 1% glutaraldehyde, postfixed with 1% osmium tetroxide, and treated routinely. The enzyme immunoassay for the detection of immunoglobulin production was applied to the media of Cell 2 by using the method of Engvall and Perlmann [1] with partial modification. The reverse transcriptase assay was performed as described previously [3].

On light microscopy, neoplastic growth was seen in the sites found by macroscopic observations and the gastric lymph nodes were also involved. Slight infiltration by the neoplastic cells was seen in the spleen. Large neoplastic cells predominated in the involved organs and smaller cells (centroblastoid and plasmacytoid cells) were admixed with them. The large cells had ovoid nuclei, moderately to highly prominent nucleoli and the relatively broad cytoplasm (Fig. 1). The cytoplasm was stained strongly with anti-gamma and light chain sera (Fig. 2), but weakly or negatively stained cells were also present. This tumour was diagnosed as an IgG-producing immunoblastic sarcoma.

The ultrastructural aspects of the cultured cells are shown in Table 1. Although the origin of Cell 1 was unknown, Cell 2 and Cell 3 were considered to be of B-cell origin owing to the characteristic structures of RER (Figs 3, 4, 5). Furthermore, IgM was demonstrated in the media of Cell 2 by the enzyme immunoassay.

Numerous intracisternal A particles were present in Cell 1 and Cell 3. Measuring between 70 and 100 nm in diameter, the particles in Cell 1 consisted of two concentric shells surrounding a relatively electron-lucent centre and were mostly attached either to the cisternal membrane of

Fig. 1. Neoplastic cells are relatively large and binuclear profiles (arrows) are seen. Diaphragmatic tumour. HE stain. $\times 400$.

RER or to another particle (Fig. 6). A few particles were present in the perinuclear spaces. Type A particles in Cell 3 exhibited morphological similarity to those in Cell 1, but the membrane of RER was frequently indistinct presumably because of a great many budding particles (Figs 7, 8). The RNA-dependent DNA polymerase activity was recognized in the media

Fig. 2. Most of the neoplastic cells stain intensely for light chain gamma globulin. Diaphragmatic tumour. $\times 250$.

of Cell 3.

Human immunoblastic sarcoma affects the superficial (especially cervical) lymph nodes [8] and a swine immunoblastic sarcoma was found in the right cheek [6]. Although immunoblastic sarcoma cells transformed from a follicular centre cell lymphoma were present in the visceral lymph nodes [5], the present case also had neoplastic lesions largely in the superficial lymph nodes. Such tumours may be liable to occur primarily in the superficial lymph nodes and multicentric form as an anatomical form may be subdivided into superficial and visceral types.

Table 1. Ultrastructure of culture cells

	Cell 1	Cells 2	Cell 3
Nuclear morphology	Often irregular	Rounded	Occasionally irregular or cleaved
Nucleoli	Moderate to large	Moderately prominent	Moderate to large
Chromatin	Slightly to moderately condensed	Slightly to moderately condensed	Slightly to moderately condensed
Organelles	RER slightly to moderately developed. Prominent Golgi complexes. Mitochondria often showing degeneration or atypical shape	RER slightly to moderately developed, rarely with parallel stacks	RER slightly to relatively well developed, occasionally forming confronting cisternae. Mitochondria often showing degeneration or atypical shape
Cell processes	A few processes	Absent	Occasionally developed

Fig. 3. Organelles are moderately developed and parallel arrays of RER are present in a lymphoid cell (arrow). Cell 2. $\times 3000$.

Fig. 4. This cell has a prominent nucleolus and abundant RER. Cell 3. $\times 7500$.

Fig. 5. Ribosomes are absent from the apposed lamellae of confronting cisternae. Cell 3. $\times 14400$.

Fig. 6. Many intracisternal A particles are present in the cisternae of RER. Cell 1. $\times 11700$.

Fig. 7. Culture cells mostly contain numerous intracisternal A particles in the cytoplasm. Cell 3. $\times 3000$.

Fig. 8. Particles stand in a line and RER is barely observed (arrows). Cell 3. $\times 14400$.

The heavy chain class switch commonly occurs from IgM to IgG to IgA and the change from IgM to IgG has been reported in a swine immunoblastic sarcoma [5]. From a mouse myeloma cell line producing IgG1, Radbruch *et al.* [9] found an IgG2b variant clone and from its variant isolated a secondary variant that had reverted to IgG1 expression. The reversion of the class switch could be explained by a sister chromatid exchange model [4]. The original neoplastic cells in our case had CIgG and the culture cells (Cell 2) secreted IgM. This may have relation to the sister chromatid exchange model.

Alternatively, Cell 2 may be derived from another clone and it is probable that type A particles contribute to establishing cell lines.

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要 約

豚免疫芽球性肉腫由来培養細胞の超微形態学的観察（短報）：門田耕一・井上 互・児玉 道・中島弘美¹⁾・荻原喜久美²⁾（農林水産省家畜衛生試験場，¹⁾茨城県県南食肉衛生検査所，²⁾麻布大学環境保健学部）—— 6 か月齢，雑種，去勢雄豚に IgG 産生性免疫芽球性肉腫がみられた。その培養細胞を電顕観察したところ，無数の槽内 A 型粒子が粗面小胞体の槽内に，少数の同様粒子が核膜腔内にあり，培養液には逆転写酵素活性が認められた。一部のリンパ球様細胞の培養液中には，IgM が存在していた。