NOTE *Avian Pathology*

Multicentric Histiocytosis Related to Avian Leukosis Virus Subgroup J (ALV-J)-Infection in Meat-Type Local Chickens

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ABSTRACT. Gross lesions characterized by swollen livers and spleens accompanied by diffuse white miliary spots, which resembled those of Marek's disease, were detected in two flocks of local meat-type chickens at a Japanese poultry processing plant in June and August 2010. The microscopic examinations revealed proliferative foci consisting of spindle or polymorphic cells in the interstitium of livers, splenic follicles and the interstitium of kidneys. These cells were positive immunohistochemically with Iba1 antibody, indicating they were histiocytic cells. Some of them contained antigens of avian leukosis virus (ALV) by immunohistochemistry,and the *env* gene of ALV subgroup J was detected from the spleens by polymerase chain reaction (PCR). Phylogenetic analysis of the PCR product indicated that the env gene might be descended from the American ADOL-7501 strain of ALV-J. These results suggest that the swollen livers and spleens of the meat-type chickens may come from histiocytic proliferation caused by ALV-J infection.

KEY WORDS: avian leukosis virus subgroup J (ALV-J), meat-type chicken, multicentrichistiocytosis.

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Avian leukosis virus (ALV) is the prototype of the genus *Alpharetroviridae* of the family *Retroviridae* and induces leukosis and sarcoma in chickens. ALV is classified into subgroups A through J by the virus neutralization test, and subgroups A, B, C, D and J are tumorigenic exogenous viruses. ALV subgroup J (ALV-J) was first detected in meat-type chickens in 1987 as a new subgroup [10]. ALV-J infection induced major economic losses with respect to meat-type chickens worldwide from the latter half of the 1980s to the early half of the 2000s, i.e., until the establishment of the ALV-J-free parental pedigree stocks. In Japan, ALV-J was detected in 1990, and major economic losses became apparent in broiler chickens in the 1990s.

We previously reported histiocytosis in broiler chickens in 12 flocks from 9 farms at the Fukushima Poultry Processing Plant in 2009 [4]. The disease was characterized by diffuse swelling of livers and spleens with miliary white spots. Histopathologically, the white spots consisted of proliferation of spindle or polymorphic cells around the Glisson's capsule of the livers and central arteries in white pulp of the spleen. These proliferating cells were determined to be histiocytic cells by immunohistochemistry. In addition, polymerase chain reaction (PCR) amplified the ALV-J *env* gene from a liver sample.

Histiocytic sarcomatosis [1, 9], reticuloma [6], multicentric histiocytosis [5, 7] and spindle cell proliferative disease [12] have been reported in chickens. It has also been shown that ALV-J infections might be related to some diseases [1, 5, 6, 9, 12].

In 2010, we encountered similar multicentric histiocytosis in a local Japanese meat-type chicken (a crossbred gamecock). Here, we describe the pathological findings of this disease and relatedness of ALV-J infection in local meat-type chickens.

A local meat-type chicken with a swollen liver and spleen was found among 800 111-day-old chickens at farm A in June 2010 at the Fukushima Meat Hygiene Inspection Office. Also, in August 2010, 3 local chickens with the same lesions were detected among 875 112-day-old chickens at farm B. In the 4 chickens, the spleens were 4 or 5 times enlarger than normal, and the livers and spleens were accompanied by diffuse white miliary spots (Fig. 1). No emaciated chickens were recognized; however, some carcasses had faded or blue pectoral muscles.

Therefore, diseased organ samples were fixed in 10% phosphate-buffered formalin or Bouin's solution and embedded in paraffin. Tissue sections 2-μm thick were stained with hematoxylin and eosin (HE) and Azan solutions. Histopathological examinations of these 4 chickens showed proliferative foci of spindle or polymorphic cells in both

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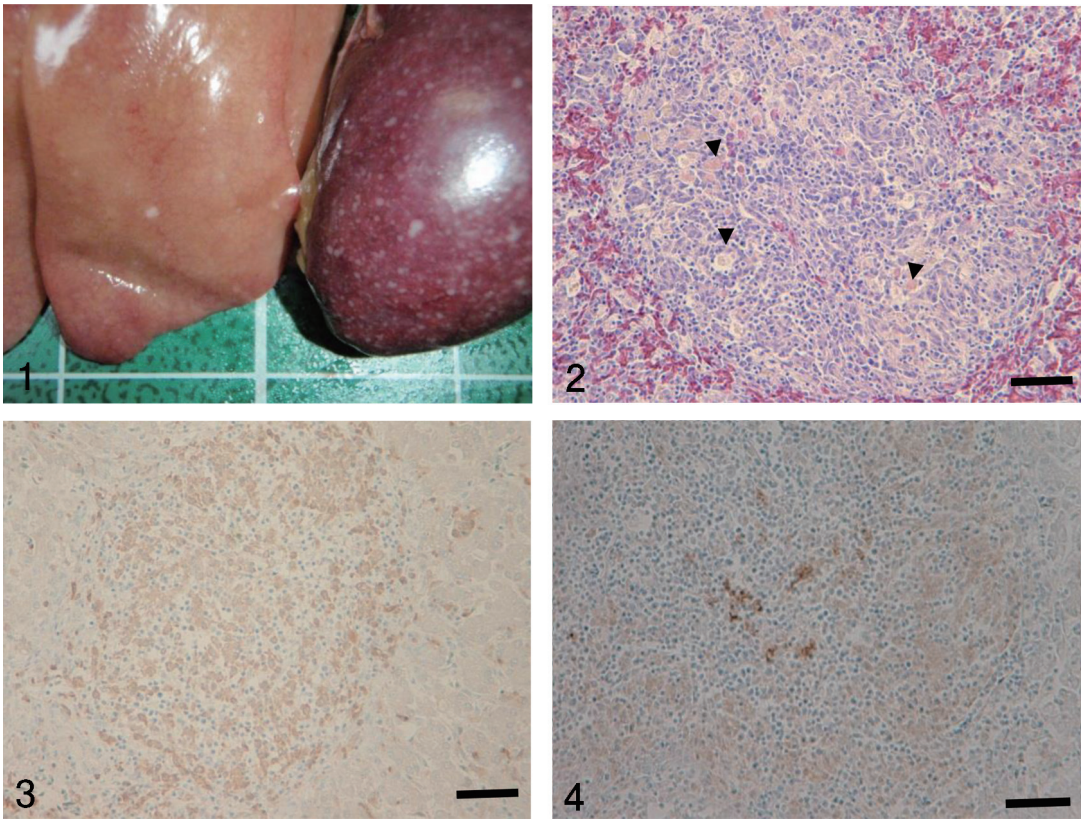

Fig. 1. The liver and spleen were swollen accompanied by diffuse white miliary spots.
Fig. 2. Spleen, HE stain, Foci of spindle or polymorphic cells with rich cytoplasm around the central artery of the splenic white pulp. Notice macrophages engulfing foreign substances (arrowheads). Bar: 50 μm.
Fig. 3. Liver, immunohistochemistry by Iba1 antibody. The spindle or polymorphic cells were stained positive for Iba1 antibody. Bar: 50 μm.
Fig. 4. Liver, immunohistochemistry by ALV antibody. Some spindle or polymorphic cells in the central part of the focal lesion were positive for ALV antigen. Bar: 50 μm.

livers and spleens. These foci were recognized around the Glisson's capsule of livers, around the central arteries and at the periphery of the sheathed arteries of splenic white pulp and also in the interstitial tissue of kidneys. In these foci of spleens, macrophages engulfing foreign substances were noticeably scattered, and lymphocytes infiltrated around the foci (Fig. 2). In the bursa of Fabricius, the numbers of lymphocytes decreased in the medulla of the lymphoid follicles; however, the structures of the follicles were maintained. In the thymus, the numbers of lymphocytes were depleted, and the boundary between the cortex and medulla was obscure. Some carcasses had faded or blue pectoral muscles in which spindle or polymorphic cells and lymphocytes were observed around the blood vessels. In the livers, collagen fibers were compressed around the foci, which were comprising of spindle or polymorphic cells according to Azan staining.

These sections of the spleens, livers and other organs were then immunohistochemically stained with Iba1 antibody against histiocytic cells (rabbit polyclonal antibody, Wako, Osaka, Japan), CD3 antibody against T lymphocyte (rabbit polyclonal antibody, Dako, Glostrup, Denmark), or anti-ALV antibody (rabbit polyclonal antibody [13]). The spindle or polymorphic cells were immunohistochemically positive for Iba1 antibody, which identified them as histiocytic cells (Fig. 3), and macrophages engulfing foreign substances were also positive for Iba1. Small lymphocytes infiltrated around these foci were immunohistochemically positive for CD3, indicating they were T lymphocytes. Also, some histiocytic cells were positive for ALV antigens (Fig. 4). Some cardiac muscle cells and the epithelial cells in the medulla of the thymus and the medulla of the foci in the bursa of Fabricius were also positive for ALV antigens. These results suggest that the histiocytic cells in the local chickens may have been caused by ALV-J infection.

The microscopical and immunohistochemical investigations of these 4 chickens indicated that proliferative foci of spindle or polymorphic cells in the livers, spleens and kidneys were composed of histiocytic cells, and some histiocytic cells contained ALV antigen in their cytoplasm. Around these proliferative foci, T lymphocytes that were

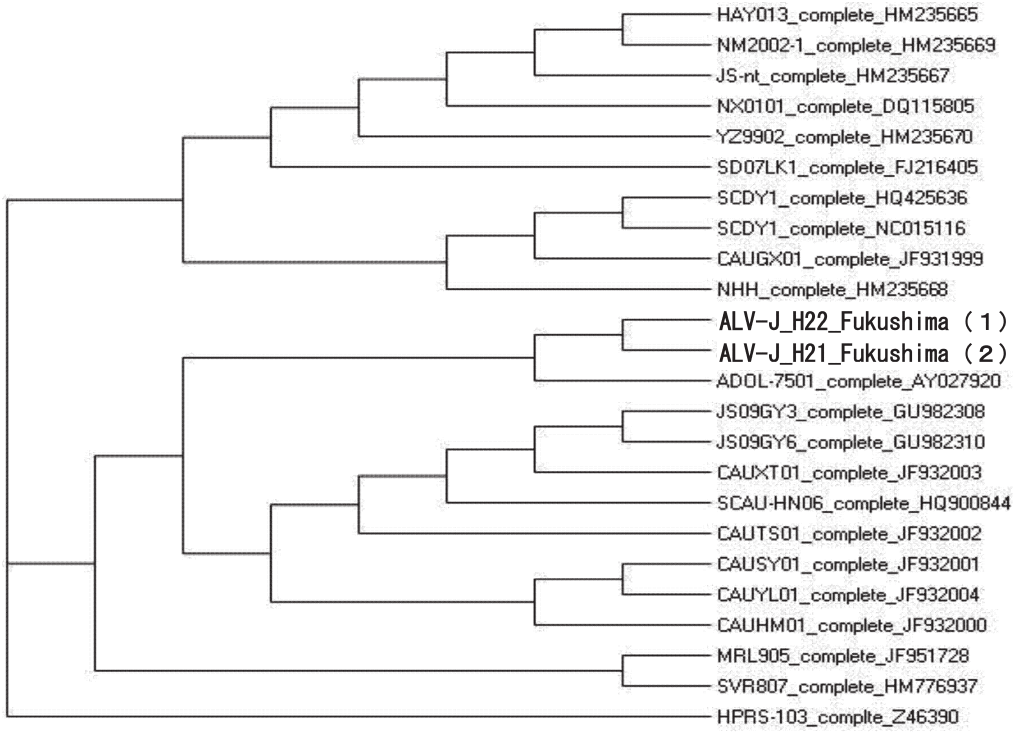

Fig. 5. Phylogenic analysis of the *env* gene of ALV-J isolated from meat-type chickens in Japan. (1) *Env* gene detected in a crossbred gamecock in August 2010(ALV-J_H22). (2) *Env* gene detected in a broiler chicken in October 2009 (ALV-J_H21).

immunohistochemically positive for CD3 infiltrated densely. These results were similar to the histiocytosis in broilers as we reported previously [4]. However, compared with the histiocytosis in broilers, the incidence was similar in the meat-type chickens, but the gross lesions of the liver and spleen with white miliary spots were milder than those of the broiler chickens.

Spleen samples from the chickens were used for purification of genomic DNA using a DNeasy Blood & Tissue Kit (Qiagen, Tokyo, Japan), and the purified DNAs were used for detection of the *env* gene of ALV-J by polymerase chain reaction (PCR) as described previously [11]. As a result, the *env* gene of ALV-J was detected from one sample. The amplified gene products were purified with a High Pure PCR Product Purification Kit (Roche, Tokyo, Japan), followed by sequencing with a BigDye Terminator v3.1 Cycle Sequencing kit (ABI) with an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, San Diego CA, U.S.A.). A BLAST search analysis showed that the *env* gene products had 97% nucleotide sequence identity to the ADOL-7501 strain of ALV-J (GenBank AY027920). The phylogenetic trees for the *env* gene was constructed by the neighbor-joining method using the sequence analysis software package GENETYX (Software Development, Tokyo, Japan) as described previously [8]. The phylogenetic tree data (Fig. 5) indicated that the *env* gene products of the Fukushima strain were molecularly close to the American ADOL-7501 strain, and clearly different from the Chinese or European HPRS-103 strains. The phylogenic analysis indicated that both H22 Fukushima (present study) and H21 Fukushima (previous diseased broiler in 2009 [4]) might be descended from a common ancestral strain, such as the American ADOL-7501 strain.

In the latter half of the 1990s in Japan, a high incidence of myelocytomatosis occurred widely in imported boiler breeders. Some cases were accompanied by a disease characterized by the systematic proliferation of foci consisting of spindle or polymorphic cells in broiler chickens, indicating the involvement of ALV-J infection [12]. Similar diseases characterized by the proliferation of histiocytic cells, likely attributable to ALV-J infection, were reported in some foreign countries, the United Kingdom [1, 3], the United States [5] and China [2], in addition to Japan [4, 6, 12]. The disease detected in meat-type chickens in this study was pathologically similar to the reported diseases, and related to ALV-J infection. Although the exact epidemiological route of the ALV-J Fukushima strains introduced in the outbreak farm was not clear, our study suggests that the ALV-J might be derived from parent broiler chickens used for crossbreeding of the crossbred gamecock. Since then, similar diseased chickens have not been detected among the meat-type chickens (a crossbred gamecock) shipped to our poultry processing plant. ALV-J-free parent chickens might be being used for breeding.

Pandiri *et al*. described that the clinical cases in chickens

occur only in individuals infected at young age with chicks being in a state of persistent viremia but not in a state of immunotolerance [9]. They additionally indicated that the amount of virus antigen (gp 85 of ALV-J) detected from these histiocytic lesions was less than that from other tumors like myelocytomatosis and that there were unique mechanisms of oncogenesis in histiocytosis [9]. Whether or not fewer ALV-J antigens are expressed in histiocytes remains to be determind.

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