

Pathological Study on Ileal Lymphoma in Swine

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Summary

In swine, the multicentric and thymic lymphomas are common, and alimentary and cutaneous lymphomas are believed to be quite rare. Recently, alimentary lymphoma in the ileum or the mesenteric lymph nodes has been described in a number of case reports. We also observed 12 cases of alimentary lymphoma affecting the ileum. In swine lymphomas, their cellular nature and characteristics have not been fully investigated for the lack of paraffin-reactive antibodies that recognize swine lymphocyte subsets and lymphoma cells. To overcome this limitation, in chapter 1, antibodies raised against human or swine lymphocytes were evaluated whether they react swine lymphocyte subsets in formalin-fixed, paraffin-embedded tissue sections pretreated with some antigen-retrieval methods. In chapter 2, based on the results of chapter 1, immunohistochemical study on swine ileal lymphomas was done together with histological, enzyme cytochemical, and ultrastructural examinations.

Chapter 1. Evaluation of Antibodies Reactive with Swine Lymphocytes in Formalin-Fixed, Paraffin-Embedded, and Antigen-Retrieved Tissue Sections.

Introduction: Typing of lymphocyte subsets in tissues and blood is important for understanding normal and pathologic immune reactions and for diagnosis of lymphoproliferative diseases in domestic animals, as well as human beings. Clarification of immune responses in swine in particular has been needed for human studies, because swine have been widely used as animal models of diseases of the cardiovascular system and skin, and of nutrition, transplantation, and malignant melanoma. Immunohistochemical labeling with monoclonal antibodies is one of the most sensitive and useful methods for such studies. Although some monoclonal antibodies that recognize swine lymphocytes have been generated, these antibodies usually require fresh cell suspensions or use of frozen tissue sections. The purposes of the study were to determine whether antibodies raised against human or swine lymphocytes are reactive with swine lymphocytes in formalin-fixed, paraffin-embedded,

and antigen-retrieved tissue sections, and to determine the conditions suitable for antigen retrieval.

Materials and Methods: A total of 33 antibodies (25 anti-human and 8 anti-swine) reactive with lymphocytes were evaluated. For antigen retrieval, we tested 11 methods: heating with 6 soaking solutions in a microwave oven, heating with 2 commercially available soaking solutions in a water bath, and enzyme digestion with 3 proteases. After these treatments, sections were immunostained along with untreated sections.

Results: Of 33 antibodies tested, polyclonal anti-human κ light chain antibody (Dako), polyclonal anti-human λ light chain antibody (Dako), CDw75 (clone LN-1), CD79 α (clone HM57), CD79 β (clone B29/123), HLA-DR (clone TAL. 1B5), and polyclonal CD3 (Dako) were strongly reactive with swine lymphoid tissues, when deparaffinized sections were digested with 0.1% trypsin (for anti-human light chain antibodies), or heated in 0.1M Tris-HCl buffer (pH 8.0) in a microwave oven or with antigen-retrieval solution (Dako) in a water bath (for the rest of antibodies). All patterns of positive staining were essentially the same as those previously described in human beings.

Conclusion: These 7 antibodies, with the antigen retrieval sequences, are helpful in identifying, distinguishing, and characterizing lymphocyte subsets in archival sections of swine lymphoid tissues. In addition, the findings indicate that some antigen-retrieval methods can appreciably expand the range of antibodies useful in paraffin-immunohistochemistry, including those that otherwise work only on sections from frozen tissues or from an antigen-derived species.

Chapter 2. Ileal Lymphoma in Swine: A histologic, enzyme cytochemical, immunohistochemical, and ultrastructural study of 12 cases.

Introduction: Although alimentary lymphomas are believed to be quite rare in swine, alimentary lymphoma in the ileum or the mesenteric lymph nodes has been described in a number of case reports. However, cellular origin and characteristics of these neoplasms have been not fully investigated. We also observed 12 cases of alimentary lymphoma affecting the ileum among 26 cases of swine lymphoma detected at meat inspection facilities in Kochi Prefecture from 1980 to 1994. In domestic animals, alimentary lymphoma is best characterized in cats and their pathological features are similar to those of Burkitt's lymphoma (BL). A study of swine lymphomas suggested that most of these cytology resembled those of BL. In addition, Epstein-Barr virus, an etiological agent of BL, has been demonstrated in a high percentage of human non-Hodgkin's lymphomas by the immunohistochemistry and the Southern blotting method. On the basis of these knowledge, we characterized ileal lymphomas in swine with pathological examinations.

Materials and Methods: We characterized 12 cases of ileal lymphoma in swine and

compared with feline and human alimentary lymphoma and Burkitt's lymphoma using histologic, enzyme cytochemical, immunohistochemical, and ultrastructural techniques. For the detection EBV, immunohistochemical examination was also done.

Results: The prevalence of ileal lymphoma in Kochi Prefecture was 0.8 per 100,000 inspected swine, and the disease was predominantly alimentary (12 / 26, 46%) or multicentric (10 / 26, 38%). None of the swine showed any symptoms such as weight loss or diarrhea. The ileal lymphomas were a solitary neoplasm arose from the Peyer's patches, along with early involvement of regional lymph nodes, and showed a characteristic pattern of follicular invasion leading to diffuse growth. The cytology of the ileal lymphomas did not resemble of that of BL, but following the lymphoma-leukemia study group of Japan (LSG) classification, 11 neoplasms were classified as diffuse lymphoma, large noncleaved cell type (follicular center cell nature) and one neoplasm was a diffuse lymphoma, mixed cell type (immunoglobulin-producing cell nature). The latter type lymphoma cells contained PAS-positive intranuclear and/or intracytoplasmic inclusions. Both types of lymphoma featured many intermingled "starry sky" histiocytes. The lymphoma cells often infiltrate into the muscular layer of the ileum in an "Indian file" pattern. Two cases also showed a characteristic transserosal metastasis into the abdomen and leukemic change. The lymphoma cells showed membrane positivity for alkaline phosphatase and diffuse cytoplasmic staining for acid phosphatase and non-specific esterase. Immunohistochemically, monoclonal intracytoplasmic immunoglobulins were shown in nine neoplasms (IgM- λ in seven, IgG- λ in one, and IgG- κ in one). The lymphoma cells of 10 cases were also positive for human pan-B cell markers; CD79 α (clone HM57) and CD79 β (clone B29/123). In the areas of follicular invasion, an attenuated network of follicular dendritic cells was visualized via an antiserum against the β subunit of S-100 protein. Ultrastructurally, strands of dilated rough endoplasmic reticulum and scattered or clustered dense bodies were noted. EBV antigen was not demonstrated in any of the cases.

Conclusion: Swine ileal lymphomas possess a distinctive pathological entity, which is a solitary neoplasm arose from Peyer's patches with early involvement of regional lymph nodes, follicular invasion leading to diffuse growth, infiltration of muscular layer in an "Indian file" pattern, transserosal metastasis into the abdomen, and predominantly large B cell type with IgM- λ type immunoglobulin expression.