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# INFORMATION

Hokkaido University confered the degree of Doctor of Veterinary Medicine (equivalent to Ph. D.) on March 25, 1997 to 12 Recipients.

The titles of their theses and other information are as follows:

## Production of monoclonal antibodies against canine leukocytes and investigation of their reactivity by flow cytometry and immunohistochemistry

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Monoclonal antibodies were produced by immunizing BALB/c mice with canine thymocytes and peripheral blood leukocytes (PBL). Ninety-nine clones out of 169 hybridomas selected by their positive reactivity with PBL in the primary flow cytometry were further analyzed by means of immunohistochemistry. In the immunohistochemical screening, some, but small in number, antibodies showed positive and constant immunoreactivity comparable with flow cytometric (FCM) findings, while the reactivity with other antibodies were not detectable or inconsistent with them.

169.1 was one of the antibodies which showed inconsistent results between FCM and immunohistochemical analyses. The antibody 169.1 reacted with non-lymphoid cells in the peripheral blood, predominantly monocytes, in FCM analysis. However, the immunohistochemical analysis showed an intense reaction with epithelial cells and neurons in the peripheral nervous system. Another intense and selective immunoreactivities were found in epithelial reticulum cells in the thymus and in sheathed arteries in the spleen. Based on the immunohistochemical and biochemical findings, 169.1 presumably recognizes a kind of cytoskeletal components which maintains the shape of cells and their processes. Immunostaining of the thymus using 169.1 demonstrated a unique region, where lacks epithelial reticulum cells, in the thymic medulla of each thymic lobule.

We acquired consistent results between FCM and immunohistochemical analyses with a monoclonal antibody which was designated 59.4. An FCM analysis using 59.4 antibody showed that the thymus contained weakly and moderately positive thymocytes, whereas the peripheral blood and spleen contained moderately and intensely positive lymphocytes. In an immunohistochemical analysis of the thymus, 59.4 labeled preferentially medullary thymocytes in moderate intensity, but a major population of cortical thymocytes were negative in reaction. In the spleen, the antibody 59.4 strongly labeled scattered lymphocytes in outer layer of the marginal zone and in the red pulp. Data from FCM and immunohistochemistry suggest that the monoclonal antibody 59.4 detects a surface antigen present on developmentally mature lymphocytes.

When adjacent sections of the thymus were stained with the anti-cytokeratin antibody that recognized epithelial reticulum cells, the unique lymphocyte aggregations without cytokeratin-

positive cells in the medulla were found to be moderately stained with 59.4 antibody as a whole. Commercially available antibodies, i.e., anti-Thy-1, anti-CD4 and anti-CD8 monoclonal antibodies, could not detect any lymphocytes within the reticulum cell-free area. At present, the following functional significance of the reticulum cell-free area is suggested. (1) The canine thymus has a region which lacks 169.1-positive reticulum cells in each thymic lobule. Therefore, the microenvironment, essential for the differentiation and maturation of thymocytes, is not uniform in the canine thymus. (2) The 59.4 antibody recognizes a surface antigen of lymphocytes selectively present in the special region without the reticulum cells.

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Original papers of this thesis appeared in "Journal of Veterinary Medical Science", Vol. 59(4), 239–244 (1997), and "Japanese Journal of Veterinary Research", Vol. 44(4), 193–206 (1997).

## Immunohistochemical study on local immune system of chicken oviduct

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The present research project was designed to evaluate the local immune status of the postnatal chicken oviduct (from one day to 78 weeks old). For this purpose cryostat sections of White Leghorn chicken's (Dekalb strain) oviduct of different ages (from 1 day to 78 weeks) were used and stained with mouse anti-chicken CT3 (anti-CD3), CT4 (anti-CD4), CT8 (anti-CD8), TcR1 ($\gamma\delta$-specific), TcR2 ($\alpha\beta$, V$\beta$1) or TcR3 ($\alpha\beta$, V$\beta$2) monoclonal antibodies for T-cell study, and HIS-C1(B-cell marker) for the study of B cell. The PLP-fixed paraffin sections were stained with goat anti-chicken IgA, rabbit anti-chicken IgG or goat anti-chicken IgM to the study of plasma cells. Bouin-fixed paraffin sections were stained with hematoxylin and eosin (H & E stain) for histological study. Point-counting method was adopted and finally two-tailed Student's t-test was used to compare the relative frequencies of different immunostained cells in the oviductal epithelium and lamina propria. T and B lymphocytes first infiltrated in the oviduct at 5 weeks after hatching. The number of T cells peaked at 15 weeks in the magnum, isthmus and uterus, while at 19 weeks in the infundibulum and vagina. The frequency of occurrence of B cells also peaked at 15 weeks from the infundibulum to the uterus (glandular part), but in the vagina (aglandular part) it did so at 21 weeks. The epithelium of the oviduct contained both granular and agranular T lymphocytes. $TcR1^+$ cells ($\gamma\delta$ T cells) were predominant in the epithelium, whereas $TcR2^+$ cells ($\alpha\beta$ T cells) were slightly more than $TcR1^+$ cells in the lamina propria. $TcR3^+$ cells were absent from the epithelium and were not numerous in the lamina propria. $CT8^+$ cells, equivalent to $CD8^+$ cells in mammals, were more numerous than $CT4^+$ cells in both the epithelium and the lamina propria. Intraepithelial B lymphocytes were very rare and exclusively located in the vagina at 19 and 21 weeks. The plasma cells first appeared in the lamina propria of the oviduct at 11 weeks of age, and their frequency peaked at 32 weeks. IgG-containing plasma cells were most numerous in the glandular part, whereas in the