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.

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

Mohammed Zahirul Islam Khan

Laboratory of Anatomy,
Department of Biomedical Sciences,
Graduate School of Veterinary Medicine,
Hokkaido University, Sapporo 060, Japan

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 ($\alpha\beta$, Vβ1) or TcR3 ($\alpha\beta$, Vβ2) 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

aglandular part IgA and IgM cells were more numerous than IgG cells. The relative frequency of T-cell subpopulations, B lymphocytes, and plasma cells was found to be higher in the vaginal part than the other parts of the oviduct.

In order to investigate the influence of sex hormones on these cells, 7-day-old chicken were injected with diethylstilbestrol (DES), DES plus progesterone (P) or sesame oil to different groups of chicken for 5 days consecutively, and killed at different time points ranging from 12 to 120 h. The oviduct, lymphoid organs (thymus, bursa and cecal tonsil), and blood of different groups of animals were collected for the observation of changes of lymphocyte population in these organs and blood. The cryostat sections of different parts of the oviduct were cut and stained with CT3, CT4, CT8, and HIS-C1, and paraffin sections were stained with IgA and IgM antisera using immunohistochemistry similar to the postnatal developmental cases. Conventional hematoxylin and eosin stain was used to study the histology of the hormone-induced oviduct and lymphoid organs. Giemsa methods were also used for the counting of lymphocyte population in the blood. Point-counting method and Student's t-test were used to analyze the infiltration of T, B, and plasma cells among different groups of chicken oviduct and in the peripheral blood.

T cells immunoreactive for CT3 first infiltrated the oviduct at 12h after the hormone treatment. Their frequency of occurrence rose from 48 to 96h. Subsequently, $CT3^+$ cells in the magnum declined in number per area, coincident with the proliferation of albuminous glands in the lamina propria, while in the vagina no decline of T cells was observed. The population of T-cell subsets in the lamina propria of both the magnum and vagina was significantly higher in the DES-treated chickens than in DES plus progesterone-treated chickens. Among T-cell subsets, $CT8^+$ cells were more numerous than $CT4^+$ cells throughout the study, this relative frequency being shared by normal adults. The first B lymphocytes and plasma cells appeared 12h and 120h after the hormone injection, respectively. Their frequency of occurrence was statistically higher in diethylstilbestrol (DES)-treated chickens than in DES plus progesterone-treated chickens. This finding suggests that like T cell, B cell infiltration to the oviduct also depends on the estrogen hormone. Depopulation of lymphocytes from the lymphoid organs, their mobilization to the circulating blood, and subsequent dynamic infiltration into the oviduct suggested that the sex hormones induced the traffic of T and B cells from the lymphoid organs into the oviduct.

The results of the present study suggest that the postnatal developmental changes of T-cell subpopulations, B cells, and plasma cells depends on different anatomical regions of the oviduct, age of the chickens, and correlated to estrogen secretion.

Original papers of this thesis appeared in "Cell & Tissue Research", Vol. 284, 317–325 (1996), "The Journal of Veterinary and Medical Science", Vol. 58, 1161–1167 (1996), and "Veterinary Immunology and Immunopathology", (1997, in press).