Distribution and Morphology of Antibody-Producing Cells in Ducks

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Abstract. Distribution and morphology of anti-horseradish peroxidase antibody-producing cells (APC) of ducks were studied by light and electron microscopic immunocytochemistry. The typical APC were plasma cells and large lymphoid cells in splenic red pulp or germinal centers. In the germinal center region, two types of positive cells were identified; the predominant positive cells in quantity were dendritic cells (DC), and the other was typical APC. In the course of cell appearance, these typical APC followed that of extragerminal center region of spleen. Other lymphoid cells in the splenic white pulp; the periarterial, the perivenous and the periellipsoidal lymphoid cells showed no evidence of antibody-production. These results suggest that some of the APC of ducks originate in the germinal centers during the course of response, and germinal centers of duck spleens and lymph nodes functions as an indispensable lymphatic element in their antibody-producing system.

One possible origin of chick plasma cells and cells of germinal centers was assigned bursa-dependent periellipsoidal lymphoid cells of splenic white pulp, and this lymphoid population was believed to be the precursor of antibody-producing cells (APC) [1]. However, some are of opinion that the ontogeny of plasma cell line begins well before that of the periellipsoidal lymphoid cells or the germinal centers. One notable point in the previous study of neonatally bursectomized ducks, there was a complete absence of periellipsoidal lymphoid cells, while the plasma cell line was present normally [13].

Duck has two pairs of lymph nodes [10] and is applied as experimental materials for studying the phylogenetic immunological relations between birds and mammals [14]. In this study immunological function of

germinal centers of duck spleen and lymph node was analysed.

Materials and Methods

Animals: Ten week-old White Pekin ducks weighing 1.5–2.1 kg were used. **Immunization:** Immunization was carried out by intramuscular or subcutaneous inoculation of 5 mg of horseradish peroxidase (HRP: type II, Sigma Chem. Co., U.S.A.) which was emulsified with 0.3 ml Freund's complete adjuvant (Iatron Laboratories, Japan) and 0.5 ml physiological saline.

Twenty-four ducks were divided into 3 groups. 1) Primary injection group; nine ducks received a primary injection at the age of 9 weeks and were sampled at 2-day intervals from 3 days after the injection until day 11. 2) Secondary injection group; eight ducks were given a booster injection 4 weeks later the first HRP injection. The ducks were sampled at 3-day intervals from 3 days later the booster injection up to day 12. 3) Control group; three ducks were injected only with Freund's complete adjuvant and four ducks were served as untreated control.

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Light microscopy: Lymphoid tissues of the spleens, the cervicothoracic and the lumbar lymph nodes were fixed in cold 95% ethyl alcohol and embedded in paraffin. Four to 5 μm sections were sliced for immunocytochemical analysis. The sections were exposed to a solution of 0.1% HRP for 30 min. The HRP binding with the anti-HRP antibody was revealed by incubation with a solution of 3,3'-diaminobenzidine tetrachloride (DAB; Merck, W-Germany) in 0.05 M tris-HCl buffer solution for 10 min [5]. The sections were counterstained with hematoxylin.

To estimate the quantitative changes of APC and plasma cells in splenic red pulp, cytoplasmimetry was applied by using ocular plotting micrometer (Olympus Optical Co., Japan) in which at 400-fold magnification an area of $2.5 \times 10^5 \mu\text{m}^2$ was contained. Number of germinal centers, APC or DC-containing germinal centers of spleens was estimated in secondary response.

Electron microscopy: Spleens and lymph nodes were fixed in 4% phosphate-buffered paraformaldehyde solution for 1 hour. Demonstration of antibody against HRP in a cell was made according to the previously reported procedure [9]. The sections were refixed in 3% phosphate-buffered glutaraldehyde solution following the reaction with HRP and DAB. Postfixation was done in 1% OsO_4 and embedded in epoxy resin.

Results

1. Cellular sequence of events occurring after the HRP injection

The cellular distribution and the sequence of events occurring after the HRP injection is described as follows and summarized in the Table 1.

1) Primary response

Spleen: A few APC were seen in the red pulp area at 3 days after the primary injection. On days 5, 7 and 9, an increased number of APC were found in the areas along the trabecular or the collecting veins. On day 11, the red pulp still contained a few number of APC.

Lymph node: Some APC were found in the peripheries of lymph nodules of lumbar nodes on day 3; the nodes consist of a pair of true lymph nodes, and the APC were detected only in the stimulated sides. On

days 5, 7 and 9, mature APC distributed in the areas surrounding the germinal centers and in the lymphatic cords. On day 11, some APC were demonstrated only in a few germinal centers of lumbar nodes.

2) Secondary response

Spleen: In the red pulp on days 3 and 6, a drastic number of APC was found and this was the most predominant during the course of this response. In histoplasmimetry, mean number of splenic plasma cells showed no quantitative change, while the APC were estimated to be 15.3% of plasma cell population on day 3, and thereafter, a sharp decrease was noted in the last course of this response. On last day 12, APC were estimated 0.1% as shown in Figure 1.

In close proximity to the central arteries, DC- and/or APC-containing germinal centers were noted. First predominant appearance of DC-containing germinal centers were found on day 3, and 90.9% of the centers contained as shown in Figure 2. Relative value of this type of germinal centers decreased with the course of this response and on day 12, nearly half of them lacked the DC. On the other hand, APC-containing centers were not frequent on day 3, and thereafter on days 6, 9 and 12, it was gradually enhanced in contrast to the declining feature of DC-containing centers (Fig. 2).

Lymph node: APC were predominantly found in the peripheral areas of the lymphatic nodules, lymphatic cords and germinal centers on days 3 to 6 (Figs. 11, 12). On days 9 and 12, APC in the extragerminal center region decreased in number in parallel with the splenic APC, while some APC in the germinal centers of lumbar nodes still showed a positive reaction.

2. Morphology of APC

APC were round to ovoid in shape and

have a voluminous cytoplasm of 6.7–13.7 μm in diameter (mean: 8.6 μm) (Figs. 3, 4). In the sections without a counterstain, nucleus

region showed no reaction deposits and mostly it located eccentrically.

These APC were identified electron micro-

Table 1. Quantitative estimation of the APC and DC in the spleens and lymph nodes of ducks during the primary and secondary responses

Lymphoid organs	Days after the primary injection					Days after the secondary injection			
	3	5	7	9	11	3	6	9	12
Spleen									
PA	—*	—	—	—	—	—	—	—	—
PV	—	—	—	—	—	—	—	—	—
PE	—	—	—	—	—	—	—	—	—
Germinal center									
APC	—	—	—	—	—	+	+	+	+
DC	—	—	—	—	—	++	++	++	+
Red pulp	+	++	++	++	+	+++	+++	++	+
Cervicothoracic lymph node									
Lymphatic nodule	—	—	++	++	+	+++	+++	++	+
Lymphatic cord	—	—	++	++	+	+++	+++	++	+
Germinal center									
APC	—	—	—	—	—	+	+	—	—
DC	—	—	—	—	—	++	+	—	—
Lumbar lymph node									
Lymphatic nodule	+	++	++	+	+	+++	+++	+	—
Lymphatic cord	—	+	++	+	+	+++	++	+	+
Germinal center									
APC	—	—	—	—	+	+	+	+	+
DC	—	—	—	—	+	++	++	+	—

PA, periarterial lymphoid tissue; PV, perivenous lymphoid tissue; PE, periellipsoidal lymphoid tissue.

* The remarks depend on the quantities of cells estimated.

—, not-detectable; +, 1–6; ++, 7–49; +++, >50/section or germinal center.

Fig. 1. Quantitative changes of the splenic plasma cells and the APC during the secondary response of ducks (Mean).

Fig. 2. Quantitative changes of the germinal centers, the DC-containing germinal centers and the APC-containing germinal centers of duck spleens during the secondary response (Mean).

scopically as plasma cells which were characterized by the reaction products in the cisternae of their rough-surfaced endoplasmic reticulum and perinuclear spaces (Fig. 5).

Subdivision of APC into two types was made by their distribution patterns and morphology. 1) Peripheral APC which distributed in the extragerminal center region and they appeared to be derived from plasma cell line. 2) APC located in the germinal center region. They appeared to originate in the lymphoid cells of the centers. The former were further divided into two subtypes in their morphology; the mature APC with voluminous strongly positive cytoplasm and immature APC with large nucleus and weakly positive cytoplasm. In the latter case, two types were also distinguished. One was the typical APC with dense cytoplasm, and only a small number of this type of APC (1-6) were detected in the area proximity to an encapsulating fibrous capsule (Fig. 6). In electron microscopy, this type of APC was assigned a large lymphoid cell which were furnishing with a round to ovoid nucleus and a few rough-surfaced endoplasmic reticulum or rich polysomes (Figs. 7, 8). The other type of positive cells were classified as dendritic cells (DC), because they were spindle in shape or had long cytoplasmic processes with weak reaction deposits on their cell surface (Figs. 9, 10). Their longitudinal length ranged between 9.8-17.6 μm (mean: 13.2 μm) and morphologically they appeared to be reticular cells. During the first two observation days of secondary response, 20-30 of DC could be demonstrated in a germinal center and showed a tendency that they appeared earlier than the typical APC.

Discussion

Immunoglobulins or antibodies in cells of avian germinal centers were demonstrated in chickens [4, 15, 18] and as an precursor cell of APC, a close ontogenic relationship between the lymphoid cells of splenic germinal centers and the plasma cell line was suggested [2]. In this study, a quantitative peak of APC in splenic red pulp was on day 3, while that of the centers was on day 12 of secondary response. Little cellular traffic between the two cell populations was acknowledged, and shown that the splenic plasma cells or APC were not the cell population derived from the lymphoid cells of centers. Further origin of splenic plasma cells or APC was studied in chickens and shown that they have a common precursor in the bursa-derived periellipsoidal lymphoid cells of spleen [1, 3, 16]. In ducks, this lymphoid cell population of spleen was also shown to be bursa-dependent as like in chickens [8, 13], however, no morphological evidence on the bursa-dependency of plasma cell line was obtained [6, 13].

In ontogenic aspect, the cells of plasma cell line were found well before the periellipsoidal one in duck and chick spleen [7, 11], and further functional results that a severe lack of the periellipsoidal lymphoid cells resulted in a complete absence of splenic APC were noted in the spleens of bursectomized ducks (unpublished data). These findings ontogenically suggest the plasma cells or APC are not the derivatives of periellipsoidal lymphoid cells. This splenic lymphoid population would rather function as antigen-recognizing cells for APC differentiation than their precursor cells. This notion is supported by the fact that the antigenic materials exclusively accumulate within the periellipsoidal lymphoid tissues infiltrating through an

endothelial wall of sheathed arteries [7,12, 19].

One of the notable points on function of germinal centers is that the mammalian's functions on quick production of antibodies as an element of their antibody-producing system when it comes into function [17], while the APC in duck germinal centers were not so predominant, but main positive cells were DC. Comparative studies on the morphological architecture of mammalian and avian germinal centers show the structural difference between the two; the former are furnished with a proper central artery in addition to the rich APC, while the latter contains only a few number of APC, and has an encapsulating fibrous capsule without a proper artery [7]. Therefore, in the immunobiological aspects, some possible functional differences between the germinal centers of two species should be considered in the phylogenetic studies of mammalian and avian immune systems.

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要 約

アヒルにおける抗体産生細胞の分布と形態：橋本善春・杉村 誠（北海道大学獣医学部家畜解剖学講座）——アヒルにおける抗 horseradish peroxidase 抗体産生細胞の分布と形態とを，光学顕微鏡および電子顕微鏡的免疫細胞学によって検討した．典型的な抗体産生細胞は脾臓赤髄ないし胚中心における形質細胞または大型リンパ球であった．胚中心域には2型の陽性細胞が区別され，そのうちで多数をしめるものは樹枝状細胞で，他は抗体産生細胞であったが，これらの抗体産生細胞は胚中心域以外に出現したものよりも遅く出現した．脾臓白髄における他のリンパ球，すなわち動脈周囲リンパ球，静脈周囲リンパ球および莢中心毛細管周囲リンパ球は抗体産生像を示さなかった．これらの結果は，アヒルの抗体産生細胞の一部は胚中心内に生じることから，脾臓およびリンパ節における胚中心は抗体産生機構の中で重要な機能をはたしていることを示していると考えられる．

Explanation of Figures

- Fig. 3. Spleen of an immunized duck 3 days after the secondary injection. Note the great number of APC in the area of red pulp; some are detectable in the venous lumen. Counterstained with hematoxylin. $\times 330$.
- Fig. 4. Spleen of an immunized duck 3 days after the secondary injection. Note the large APC with round to ovoid configurations. Germinal center (GC) contains some DC. Without counterstain. $\times 330$.
- Fig. 5. Electron micrograph of APC in the red pulp of an immunized duck spleen 3 days after the secondary injection. Note the dense reaction product in the cisternae of each rough-surfaced endoplasmic reticulum and perinuclear space. The APC is characteristic of a mature plasma cell. $\times 12,000$.
- Fig. 6. Germinal center of a spleen 12 days after the secondary injection. Note a few large APC within the germinal center region. Counterstained with hematoxylin. $\times 730$.
- Fig. 7. Electron micrograph of a lymphoid cell in the germinal center. Note the moderately developed Golgi-apparatus, some slightly distended rough-surfaced endoplasmic reticulum and numerous polysomes. $\times 8,100$.
- Fig. 8. Low powered electron micrograph of a splenic germinal center. Note the large population of medium- or large-sized lymphoid cells. They are demarcated by a thin fibrous capsule from the periarterial lymphoid tissue. $\times 2,400$.
- Fig. 9. Germinal center of an immunized duck spleen 3 days after the secondary injection. Note the cells with long dendritic patterns bearing positively reacted materials on their cell surface. Without counterstain. $\times 720$.
- Fig. 10. Germinal center of an immunized duck spleen 6 days after the secondary injection. Note the considerable number of intensely reacted DC among the lymphoid cells. Counterstained with hematoxylin. $\times 330$.
- Fig. 11. Germinal center in lumbar lymph node of an immunized duck 3 days after the secondary injection. Note the great number of APC. In the germinal center region, DC and APC are simultaneously found. In the extragerminal center region, the APC tend to be located in the areas of the lymphatic nodule (LN), lymphatic cords (LC) and in the areas surrounding the germinal centers (GC). Without counterstain. $\times 250$.
- Fig. 12. Germinal center in the lymph node of an immunized duck 6 days after the secondary injection. Note the DC in the area of germinal center and APC in the surrounding area of the lymphatic nodule. Counterstained with hematoxylin. $\times 330$.