Intranodal Pathway of Phagocytes Ingesting Foreign Materials in Ducks

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ABSTRACT. Intrasinus free phagocytes ingesting injected colloidal carbon (CC) or alum-precipitated bovine serum albumin (AP-BSA) migrated through the lymphatic cords into the lymphatic nodules, and those with CC became situated in clusters around or inside the germinal centers. Horseradish peroxidase (HRP) also showed a similar intranodal pathway but often mediated by the reticulum cells in the lymphatic cords and nodules as well as the free phagocytes. This confirms the sequential cooperation of free macrophages and regional lymphocytes during the initial stages of the intranodal immune response.

The cooperation of macrophages and lymphocytes during various immune responses such as maturation and differentiation of T lymphocytes [11] and lymphocyte proliferation [1, 14] has been studied. Mosier [10] found that antibody forming-cells cluster around macrophages in the primary response to sheep erythrocytes in vitro. Lipsky and Rosenthal [9] reported the occurrence of antigen-mediated physical interactions between lymphocytes and syngeneic macrophages in the lymph nodes of immunized guinea pigs.

In the present study, the intranodal pathway of duck phagocytes was analyzed at intervals by histoplanimetry in relation to the distribution of T and B lymphocytes as shown by Sugimura et al. [16] after subcutaneous injection of different foreign materials.

MATERIALS AND METHODS

**Animals:** 150 White Pekin ducks aged 6 to 10 weeks after hatching were bred.

**Administration of foreign materials:** A 1:10 dilution of colloidal carbon (CC) solution (Perikan Ink c11/1413a, Günter, Wagner, Germany) in physiological saline (0.05 ml) was injected in subcutaneously into each web. Alum-precipitated bovine serum albumin (AP-BSA) was prepared from crystalline BSA (Sigma Chemical Co., U.S.A.) by the method of Katsura [6] and 0.25 mg of AP-BSA in 0.05 ml of physiological saline was injected subcutaneously into each web. 5 mg of horseradish peroxidase (HRP: type II, Sigma Chemical Co., U.S.A.) in 0.05 ml of physiological saline was administered into the paddle of the ducks. Four or 5 ducks each from differently treated groups were sacrificed at 10 min to 4 weeks after treating. As controls non-treated ducks were also sacrificed.

**Histology and histoplanimetry:** Lumbar lymph nodes were fixed in Bouin's fluid (CC-group) or 95% ethanol at 4°C (BSA-group) and embedded in paraffin. 4 μm-sections were made and stained with hematoxylin-eosin (CC-group) and hematoxylin (BSA-group). Some semithin sections were also prepared from the tissue embedded in epoxy resin and stained with toluidine blue. Phagocytized AP-BSA was detected by indirect immunohistochemistry using rabbit anti-BSA serum (Miles-Yeda Ltd., Israel) after the elimination of the endogenous peroxidase on the tissue sections. As controls sections were then treated with normal rabbit serum. To estimate the area of lymphatic sinuses,
cords and nodules, the point-counting method [15, 17] was applied under a microscope using an ocular plotting micrometer (Olympus Optical Co., Japan). The number of phagocytizing cells were counted with an unit area (CC-group: 0.28 mm², AP-BSA-group: 0.16 mm²). The phagocytes with CC or AP-BSA in the germinal centers were excluded from the counting because of the uncertainty of their appearances.

Electronmicroscopy: Tissues from HRP-group were fixed in phosphate buffered 3% glutaraldehyde at 4°C for 2 hr and sliced 40 µm thick were made with a tissue sectioner (Sorvall, TC-2, U.S.A.). After treating with 3, 3’-diaminobenzidine tetrahydrochloride in 0.05 M tris HCl buffer solution (pH 7.6) [4] for 1 hr, the tissues were postfixed with 1% osmium tetroxide for 2 hr and embedded in epoxy resin. Observation were made using HU-12A electron microscope (Hitachi).

RESULTS

At 10 min after local injection, CC, AP-BSA or HRP were detected in the lymphatic sinuses of the lumbar lymph nodes (Figs. 4, 5, 6). Intrasinus phagocytes had small or medium-sized phagosomes. Occasionally some granulocytes were seen in the lymphatic sinuses at 10 or 30 min after injection. At this stage some phagocytes were migrating between the endothelial cells of the sinuses, and then into the cords. Some phagocytes with CC or AP-BSA were located in the lymphatic cords at 10 or 30 min, respectively after injection (Fig. 7). In the case of HRP, however, some sinus endothelial cells with small phagosomes were also phagocytic within 1 hr after injection (Fig. 6). With a time lapse of the injection, HRP was also detected in the reticulum cells of the lymphatic cords or nodules, being distributed diffusely almost throughout the cytoplasm at 2 hr. During this observation period, migrating phagocytes were seen neither in the lymphatic nodules nor in the germinal centers.

At 3 or 6 hr after injection, the injected materials were detected within the lymphatic nodules. In the case of CC, phagocytes increased in number with time, with phagosomes increasing in size but decreasing in number as compared with those at 30 min to 1 hr after injection. At 1 day after injection, many phagocytes with AP-BSA were found in the lymphatic cords and nodules, though their frequency was individually variable. The histoplasmimetry of phagocytes with ingested CC or AP-BSA in different parts of nodes were shown in Fig. 1 & 2. HRP was also transported from the lymphatic cords to the nodules by free phagocytes, but at 4 to 6 hr after injection, it was mostly found in the reticulum cells of the lymphatic cords and nodules (Fig. 8), and these reticulum cells had their processes extending to adjacent lymphocytes (Fig. 9). The intracellular distribution of HRP was most prominent within 12 hr after injection, being poor at 24 hr after injection.

At 3 days after injection, the phagocytes with CC or AP-BSA accumulated in the lymphatic nodules occasionally forming some phagocyte islet-like cell clusters. At 1 week after injection, the accumulation of phagocytes in the lymphatic nodules became more prominent, showing two types of clusters, one of crescent-shape in the marginal zone and the other of tubercle-shape in the deep zone (Fig. 10, 11). In these phagocytes in clusters, some phagosomes fused with each other and occasionally the whole cytoplasm was occupied with a single large phagosome containing carbon particles.

During 1 week after injection, some phagocytes with CC were located around the periphery of the germinal centers and occasionally within the germinal centers (Fig. 12).

At 3 or 4 week after injection, phagocytes with CC were still aggregated in enlarged clusters.
Fig. 1. Number of CC ingesting phagocytes in the duck lumbar lymph node

Fig. 2. Number of AP-BSA ingesting phagocytes in the duck lumbar lymph node
DISCUSSION

In the present study, the foreign materials were shown to be ingested by phagocytes in the lymphatic sinuses. Then these phagocytes migrated into the lymphatic cords through the intercellular junction of sinus endothelium, and they seemed to proceed to the lymphatic nodules to have a close contact with lymphocytes and reached occasionally the germinal centers.

Sugimura et al. [16] examined histologically the thymus (T)- and/or bursa of Fabricius (BF)-dependent areas of the lymph nodes of chemically bursectomized, surgically thymectomized and/or X-ray irradiated ducks, revealing that the lymphatic nodule represented the T-dependent area while the lymphatic cord and possibly the germinal center the BF-dependent one. In the present results, it was shown that phagocytes with CC first entered the BF-dependent lymphatic cord and then moved the T-dependent lymphatic nodule and finally, they reached the possibly BF-dependent germinal center. Such an intranodal pathway of phagocytes is summarized in Fig. 3.

A similar intrasplenic migration was also followed with $^{125}$I-human serum albumin-bearing phagocytes in the chick spleen [18] of which T- and BF-dependent areas were confirmly represented. The cells were initially found in the BF-dependent perilipsoidal lymphoid tissue [5] moving toward the T-dependent periarterial lymphoid area [2] with a time lapse after the injection. The labelled antigen-bearing phagocytes were finally most conspicuous in clusters in the BF-dependent germinal center [2], in which immature pyroninophilic lymphocytes were encircled by a thin fibrous capsule.

It is almost comparable with the intranodal pathway of mammalian macrophages. Kotani et al. [7, 8] showed that the migratory macrophages with CC first appeared in the lymph sinuses and next in the deeper cortex at the corticomedullary junction or the medullary cord near follicles and finally entered the germinal centers only through their medullary pole. In the lymph nodes of some mammalian species, the corticomedullary junction and the medullary cord has been believed to be of T- and bone marrow (BM)-dependent area respectively [3, 12, 13] through which CC-bearing macrophages pass to the BM-dependent germinal center [13].

The systematic migration of phagocytes commonly observed in avian and mammalian lymph nodes, namely first BF- or BM-dependent area or/and next T-dependent area and finally BF- or BM-dependent area, seems to have an important immunological significance, suggesting an indispensable interaction between the antigen-
presenting phagocytes and lymphocytes during the first stage of antigen recognition. Though duck lymph nodes have a primitive structure as compared to those of mammalian species, such homology of the intranodal migration of phagocytes related to different population of lymphocytes is of interest. The duck lymphoid tissues are considered to be well differentiated in functions possessing an almost equivalent clearance and antibody-producing activities against foreign materials as in mammals. Further ultrastructural studies are needed to clarify the immunological meanings of the intranodal migration, in particular the cluster formation of antigen-presenting phagocytes in the lymphatic nodules or germinal centers.

REFERENCES


EXPLANATION OF FIGURES

Fig. 4. Intrasinus phagocytes containing numerous phagosomes of carbon particles. 10 min after injection. LS: lymphatic sinus. Toluidine blue. ×690.

Fig. 5. AP-BSA ingesting phagocytes in the lymphatic sinuses and those without AP-BSA in the lymphatic cords (LC). 10 min after injection. Immunocytochemistry and hematoxylin. ×440.

Fig. 6. Intrasinus phagocytes and sinus endothelial cells ingesting HRP within medium- or large-sized phagosomes (arrows). 10 min after injection. ×3,300.

Fig. 7. Phagocytes with CC moving from the lymphatic sinus into the cord (arrows). 10 min after injection. Toluidine blue. ×460.

Fig. 8. HRP in the long-processed reticulum cells (arrows) of a lymphatic nodule in proximity to a germinal center (GC). 6 hr after injection. Cytochemistry and toluidine blue. ×440.

Fig. 9. HRP-containing reticulum cells extending long cytoplasmic processes between neighbouring lymphocytes in the lymphatic cord. 4 hr after injection. Electronmicroscopic cytochemistry. ×4,800.

Fig. 10 & 11. The crescent (Fig. 10)- and tubercle (Fig. 11)-shaped aggregates of phagocytes ingesting CC in the marginal and deep areas, respectively, of a lymphatic nodule. 1 wk after injection. Hematoxylin-eosin. ×160.

Fig. 12. Small clusters of phagocytes with CC around and inside (arrows) the germinal center. 4 wk after injection. Hematoxylin-eosin. ×690.

要約

異物投与後のアヒルリンパ節内の食細胞移動経路: 中村和男・橋本善幸・北川浩・工藤規雄（北海道大学薬学部薬剤製剤学教室）——アヒル皮下に可消化性カーポン又は明礬沈殿ウシ血清アルブミンを投与すると、脣リンパ節リンパ洞内遊走性食細胞はこれらの物質を摂取後リンパ索よりリンパ小脳に入り、可消化性カーポンを摂取した食細胞は食細胞小島を形成しつつ最終的には胚中心周囲域あるいは胚中心内に到達した。また細胞由来ペルオキシダーゼでは上記の食細胞を介する細胞内移動のほか、リンパ洞内皮および実質内細胞間細胞を介する移動もみられた。これらの事実は食細胞とリンパ球間の協調がリンパ節内における初期免疫応答時に重要であることを示すと思われる。