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ULTRASTRUCTURE OF THE LUMINAL IMMUNOCOMPETENT CELLS OF THE CHICKEN'S AIR PASSAGEWAYS

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SUMMARY

The morphological peculiarities of immunocompetent cells that were encountered in bronchopulmonary lavage fluids of the Leghorn chickens were investigated at the electron microscopic level. Four distinctive cell types; namely heterophils, eosinophils, lymphocytes, and macrophages, the latter cells predominated. Luminal heterophils exhibited a wide range of ultrastructure heterogeneity, which were strongly suggestive for both active phagocytic and secretory functions. Luminal eosinophils were recognized mainly through their extremely elongated rod-like electron dense cytoplasmic granules. The outer contour of the luminal eosinophils was mostly broken by comparatively large pseudopodia, and their cytoplasm were packed with many vacuolar and vesicular structures. The cytoplasm of luminal lymphocytes was characterized by abundant ribosomes, few rER tubules, and electron-dense membrane-bound granules. Such criteria were clearly suggestive for a well-developed secretory nature for luminal lymphocytes. Luminal macrophages represented the predominant cell type. Their ultrastructure features were strongly suggestive for an active phagocytic function within the lumen of the chicken's air passageways.

INTRODUCTION

The lumina of the chicken's air passageways contain a wide variety of migrating immunocompetent cells capable of mounting an effective immune response against various air-born antigens.

As being found on the inner surfaces of the respiratory tract, they come in contact with particles and pathogens contained in the inspired air. They are also exposed to higher oxygen pressures than their counterparts elsewhere in the body. Their mobility, phagocytic capacity, and bactericidal

properties are essential to the maintenance of clean and sterile alveoli (Bowden, 1973).

Beside their immunological role, studies of these cells are of interest because their migratory patterns, phagocytic behavior, and secretory potential are pivotal events in the pathogenesis of pulmonary diseases. Thus, although pulmonary macrophages are an essential line of defense against inhaled infectious and non-infectious particles, they can also injure the host while exercising their defensive role (Allison, 1977). In this respect, pulmonary macrophages have been reported to play an effective role in establishing chronic pulmonary diseases such as tuberculosis (McDonough et al., 1993).

Luminal immunocompetent cells migration is mediated through the production of specific migration enhancement factor. Weisbart et al. (1974) were the first to propose that migration enhancement factor is a lymphokine.

Several studies had been devoted to clarify the morphology and functions of the chicken's immunocompetent cells (Enbergs, 1973a; Enbergs, 1973b; Ericsson & Nair; 1973; Maxwell, 1973; Macrae & Spitznagele, 1975; Evans et al., 1995; Karmakar & Kumdu, 1995; Kogut et al., 1995a; Kogut et al., 1995b; Kulessa et al., 1995; Trigoso & Stockert, 1995; Andreasen et al., 1996; Merrill et al., 1996; McNagny et al. 1996; Terashima et al., 1996).

Most of the aforementioned investigations had been carried out on circulating immunocompetent cells. No studies in the available literature have been devoted to elucidate the ultrastructural peculiarities of the luminal immunocompetent cells especially those of the chicken's air passageways.

Therefore, the objective of the present study is to elucidate the different immunocompetent cells that might exist within the lumina of the chicken's air passageways as well as to clarify the presumed functional roles for such cells as reflected from their ultrastructural peculiarities.

MATERIAL AND METHODS

The present study was conducted on 50 leghorn chickens of different ages ranging from 1 to 4 months. Birds were anesthetized and intratracheally injected with phosphate buffered saline. The recovered saline was mixed with an equal amount of general electron microscope fixative composed of 5% glutaraldehyde, 3% formaldehyde in 0.05 M phosphate buffer at pH 7.4. Cells were post fixed in osmium, dehydrated in a graded series of ethanol, and embedded in poly/Bed 812. Thin sections were cut using ultratome, and were doubly stained with aqueous lead citrate and aqueous uranyl acetate and examined with transmission electron microscope (Hayat, 1989).

RESULTS

Ultrastructural examination of the prepared sections from the recovered broncho-pulmonary lav-

age fluids revealed the presence of four distinctive cell types; namely heterophils, eosinophils, lymphocytes and macrophages (Fig. 1). The latter cells predominated.

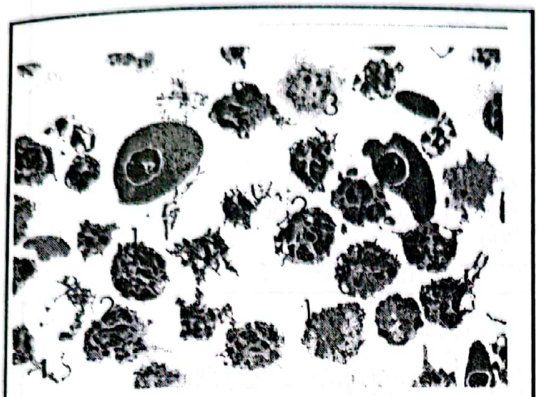

Figure (1): Low resolution of the luminal immunocompetent cells in the chickenís air passageways. Note, heterophil (1), Eosinophil (2), and lymphocyte (3) X 8, 400.

The heterophils (Fig. 2) appeared as irregularly spherical cells with short pseudopodia and random membranous folds. The polymorphic nucleus consisted of two to five lobes. The nuclear chromatin was of the condensed type, and frequently clumped along the inner surface of the nuclear envelope. The center of each lobe had a small amount of euchromatin. The nucleolus was indistinct. The cytoplasm enclosed a large number of membrane-bound specific granules which were differentiated into two distinctive forms: large electron-dense granules; and comparatively smaller electron-dense granules possessing an electron-lucent central core. Few organelles such as spherical mitochondria, scarce rER tubules, and small Golgi complex were also encountered especially within the perinuclear region. Within the cytoplasm a considerably large number of randomly scattered vesicular structures of variable size and electron density were frequently seen.

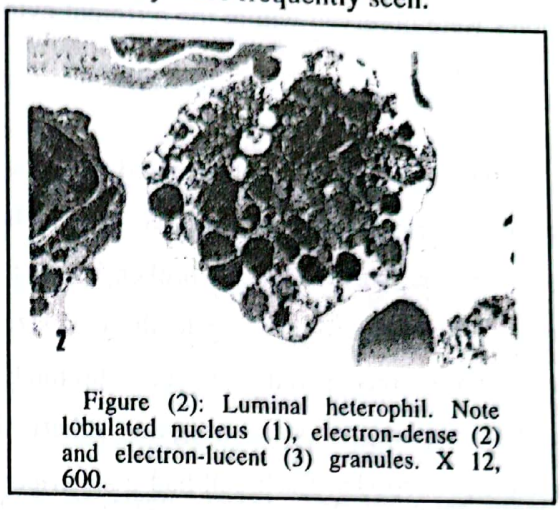

Figure (2): Luminal heterophil. Note lobulated nucleus (1), electron-dense (2) and electron-lucent (3) granules. X 12, 600.

The eosinophils (Fig. 3) appeared as spherical cells with irregular outer contour and large peripheral pseudopodia. The nucleus was mostly bilobed with dense peripherally clumped chromatins and a distinct nucleolus. The cytoplasm was abundant and showed many small spherical mitochondria, small Golgi complex, free ribosomes, and a considerable number of rER tubules. The specific granules were membrane-bound, spherical, ovoid or extremely elongated-rod-shaped structures.

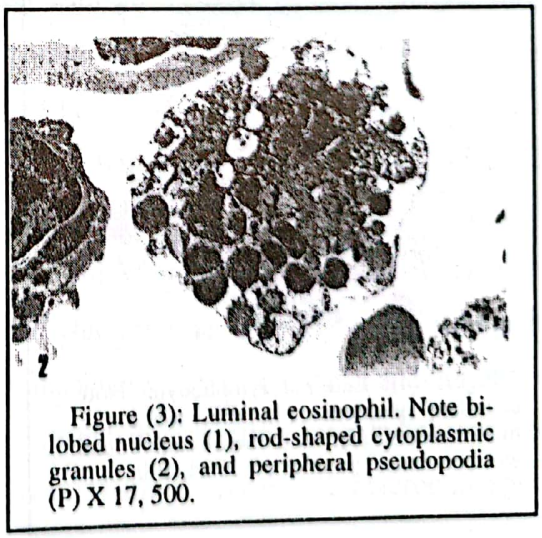

Figure (3): Luminal eosinophil. Note bilobed nucleus (1), rod-shaped cytoplasmic granules (2), and peripheral pseudopodia (P) X 17, 500.

The granule matrix was homogeneous electron-dense. Some electron-lucent membrane-bound vacuoles were observed within the different cytoplasmic regions.

The lymphocytes (Fig. 4, 5, 6) appeared mostly as irregular spherical cells of variable size. The spherical outline was frequently broken by pseudopodial projections. According to the cell size, three types were recognized. The small and medium-sized lymphocytes were irregular spherical with short peudopodia. Each cell had a spherical, large, centrally located nucleus. The nuclear chromatin was condensed peripherally. The core of each nucleus had a considerable amount of euchromatin, and frequently showed a distinct nucleolus. The major part of the cytoplasm was occupied by dense ribosomal population. Besides, relatively few other cytoplasmic organelles were present such as mitochondria, and few rER tubules. Large lymphocytes (Fig. 4, 5) were infrequently observed.

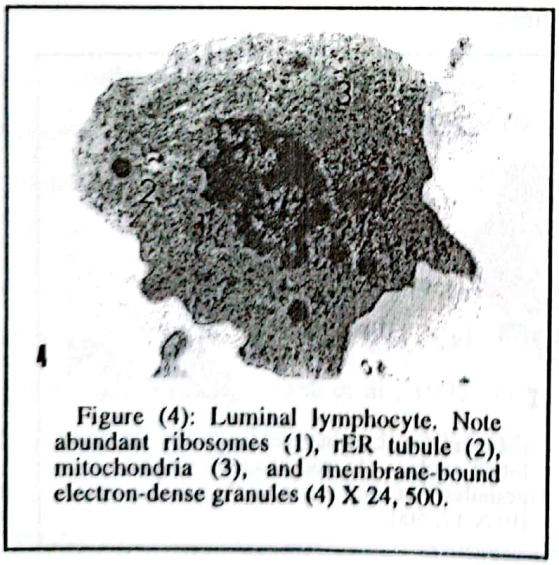

Figure (4): Luminal lymphocyte. Note abundant ribosomes (1), rER tubule (2), mitochondria (3), and membrane-bound electron-dense granules (4) X 24, 500.

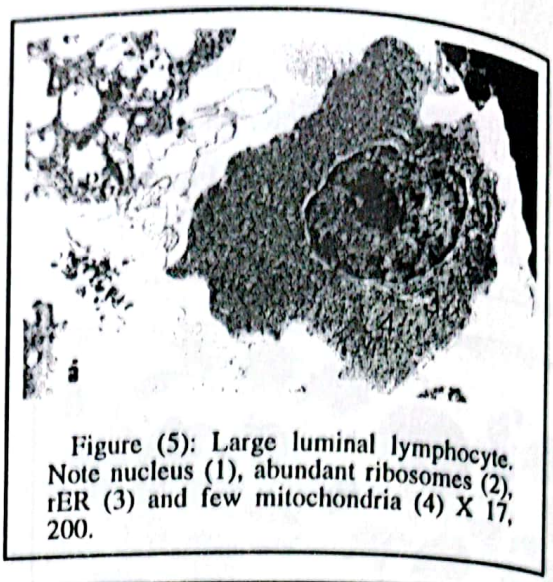

Figure (5): Large luminal lymphocyte. Note nucleus (1), abundant ribosomes (2), rER (3) and few mitochondria (4) X 17, 200.

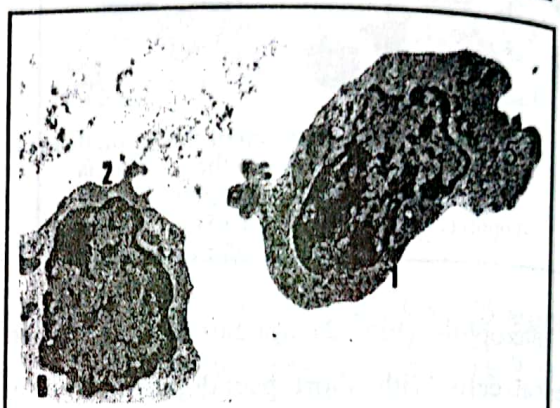

Figure (6): Medium-sized (1) and small luminal lymphocytes (2). Note nucleus (3), few mitochondria (4) and abundant ribosomes (5) X 17, 500.

They had a comparatively larger amount of ribosome-rich cytoplasm. Other organelles were scarce and consisted of few mitochondria and rER tubules as well as few membrane-bound electron-dense granules.

The macrophages (Fig. 7, 8) represented the predominant cell type. They appeared as irregularly spherical cells with long peripheral pseudopodia. The nucleus was indented, and was located peripherally. The nuclear chromatin was mostly of euchromatic type with thin film of heterochromatin attached to the inner surface of the nuclear en-

velope. One or more nucleoli were found centrally. The abundant cytoplasm was equipped with a well-developed organelles and inclusions. The organelles included many spherical and elongated mitochondria, a considerable number of free ribosomes, few rER tubules, and a well-developed Golgi complex. Considerable number of primary and secondary lysosomes were recognized. Primary lysosomes were identified as electron-lucent vesicles. Secondary lysosomes were represented by a comparatively large electron-dense vesicles of variable size. Few electron-dense membrane-bound granules were seen mostly close to the cell surface (Fig. 8).

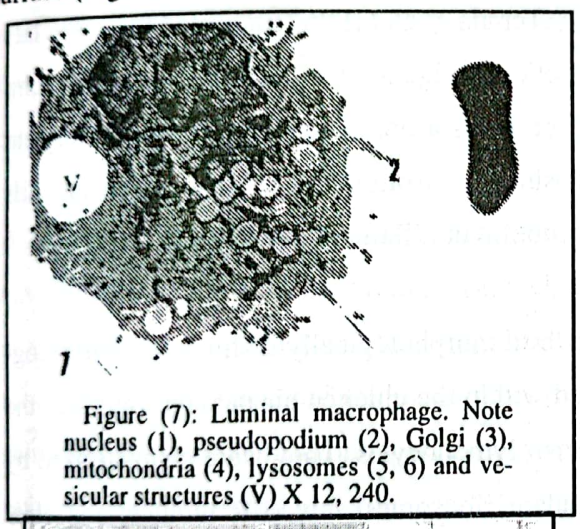

Figure (7): Luminal macrophage. Note nucleus (1), pseudopodium (2), Golgi (3), mitochondria (4), lysosomes (5, 6) and vesicular structures (V) X 12, 240.

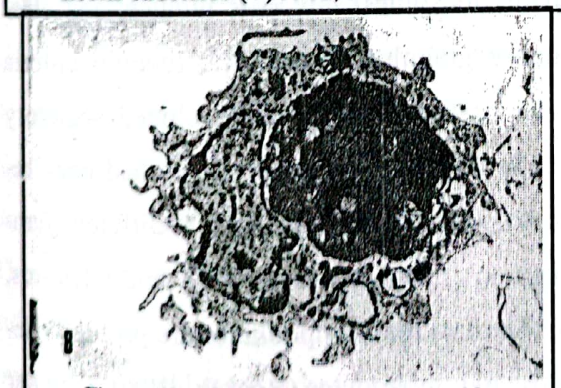

Figure (8): Luminal macrophage. Note nucleus (1), mitochondria (2), primary lysosome (L) heterophagosome (3), rER tubule (4) and electron-dense membrane-bound granule (G) X 12, 240.

DISCUSSION

Luminal immunocompetent cells that had been described in the present study included four distinctive cell types; namely heterophils, eosinophils, lymphocytes, and macrophages.

The ultrastructure criteria of the luminal heterophils that had been described in the present study were comparable to those previously described for circulating heterophils (Ericsson & Nair, 1973; Maxwell, 1973). The irregular spherical shape and the presence of pseudopodia suggested motile cells that were actively engaged in phagocytosis. The phagocytic nature of heterophils had been well-documented by earlier studies. Hirsch (1962) reported that heterophils are the first cells to enter the tissue from blood vessels . In this respect, Kogut et al. (1995a and b) in one day-old chicks reported an increased influx of inflammatory heterophils following intraperitoneal administration of live Salmonella-immune lymphokines (ILK) with no increase in macrophages. Moreover, significant increases in adherence, chemotaxis, and phagocytosis of S. enteritidis were found with such lymphokines treated chicks . The same authors added that prophylactic treatment of one day-old chicks with ILK involves activated heterophils which migrate rapidly to the inflammatory stimulus where they phagocytize and kill bacteria. The present investigation revealed the existence of two distinct types of heterophil specific

granules. Similar findings were reported for Gallus (Ericsson & Nair, 1973) and for other domestic avian species (Maxwell, 1973). The presence of acid hydrolase enzyme has been demonstrated histochemically (Topp & Carlson, 1972 a & b) within some of the heterophil granules, suggesting that they are lysosomal structures. Evans et al. (1995) had demonstrated that chicken heterophils specific granules are the source of active antimicrobial peptides designated CHP1 and CHP2. Such peptides are effective in reducing the survival of some avian pathogens such as Candida albicans, Salmonella enteriditis, Salmonella typhimurium, Campylobacter, Escherichia coli,, and Bordetella avium.

The ultrastructural morphology of the eosinophils demonstrated in the present study were to a great extent similar to that previously described for other avian species (Maxwell 1978; Maxwell & Siller, 1972). The functions of the avian eosinophils are not clearly understood. In mammals, eosinophils had been reported to occur in the tissues for much of their life span and interact with immune complex, frequently by active phagocytosis (Cline, 1975). The large pseudopodia described in the present study, together with the clear vacuoles recognized within the eosinophil cytoplasm might substantiate the presumed phagocytic function of the avian eosinophils. However, it is not quite clear the exact target to which such phagocytic activity is directed either toward elimination of antigen-antibody complex and / or toward airborn pathogens. Adachi et al. (1995) demonstrated that the process of eosinophils activation is largely dependent on certain cytokines which were released under certain pathological conditions such as hypersensitivity. Terada et al. (1996) had the opinion that eosinophils might be activated during the journey from blood stream to inflammatory tissues by the adhesion to endothelial cells and fibronectin. Moreover, Burke et al. (1996) reported that certain chemokines such as eotaxin may facilitate eosinophils migration from blood vessels in the lung by increasing eosinophils adhesion to lung microvascular endothelial cells. Terada et al. (1996) suggested that very late activation antigen-4 (VLA-4) on the eosinophil surface plays a prominent role in the recruitment of eosinophils from blood vessels and eosinophils locomotion in inflammatory tissues.

The third morphologically distinct cell line recognized within the chicken air passageways was the luminal lymphocytes. They were characterized by abundant ribosomes, few rER tubules, and electron-dense granules. Such ultrastructural criteria were suggestive for a well-developed secretory nature. In this respect, lymphocytes had been reported to produce as many as 100 different lymphokines (Altman & Katz, 1982 and Klesius, 1982). The fact that lymphokines are primarily secreted by T-lymphocytes (Rocklin, 1980) together with the presence of an electron-dense granules

revealed in the present work might suggest that luminal lymphocytes are of T-cell line. Such postulation might gain additional support by the observation of Doughlas (1983) that in peripheral blood, there are about 70% T-cells and 20% B-cells, and the remaining 10% probably constitutes null cells.

The electron-dense granules recognized in the present study may represent true secretory granules rich in biologically active secretory products such as lymphokines, or they had merely represented some forms of lysosomes. Relevant to this postulation, Grossi and Greaves (1981) asserted that a small proportion (5-15%) of T-cells cotains few granules of variable electron density which correspond to azurophilic granules.

Macrophages represented the fourth cell line that was characterized within the lumina of the chicken air passageways. General speaking, pulmonary macrophages had been differentiated into three main classes: alveolar macrophages; airway macrophages and interstitial macrophages (Bowden, 1973). Airway macrophages may be present as passengers on the mucous escalator or may be found beneath the mucous lining adherent to the bronchial epithelium (Sorokin & Brain, 1975). These airway macrophages most likely are the result of alveolar-bronchiolar transport of alveolar macrophages, although it has been suggested that they are the product of direct migration of cells through the bronchial epithelium (Brundelet, 1965 and Kilburn, 1974).

The cytoplasm of the luminal macrophages revealed in the present study was equipped with a large number of lysosomes, phagosomes, and other vesicular structures showing variable degrees of electron density. In addition, the spherical contour of the cells was in most cases broken by a considerable number of large pseudopodia. Such morphological features were strongly suggestive for an active motility and phagocytic function. Airway macrophages are usually credited with keeping the surfaces of the air passageways clean and sterile. They ingest inhaled pathogens and particles as well as endogenous effete cells and even "worn-out" surfactant (Eckert et al., 1983; Bowden, 1973; Bowden & Adamson, 1978; Brain, 1970; Brain et al., 1977 and Hocking & Golde, 1979 a & b). The phagocytic and lytic potentials of pulmonary macrophages provide most of the bactericidal properties of the lungs. Moreover, they are also responsible for ingesting and killing viruses (Rose et al., 1982; Rose et al., 1983). Sorokin (1983 a & b) used electron microscopy and histochemistry to describe the activation of macrophages and the dynamics of lysosmal elements after exposure to iron oxide aerosols. The lysosomes attach themselves to the phagosomal membrane surrounding the ingested

pathogens. Then the lysosomal and phagosomal membranes become continuous and the lytic enzymes kill and digest the bacteria. Macrophages are responsible for the intracellular killing of parasites such as African trypanosomes and malarial parasites (Sethi, 1982).

More important to the microbicidal activity of macrophages that lytic enzymes are the oxygen-dependent cytotoxic systems. Phagocytosis triggers increased oxygen consumption and the generation of oxygen radicals such as superoxide which modify macromolecules of pathogens (Klebanoff, 1980). Virulent pathogens may elude the microbicidal mechanisms of airway macrophages and gain access to the lymphatics or the bloodstream to establish infection (McDonough et al., 1993).

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