Electron Microscopy of the Irises of Chickens with Spontaneous Ocular Leukosis

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SUMMARY

The irises of seven chickens with lesions of spontaneous ocular leukosis were examined by light and electron microscopy. Histologically, the abnormal contents of the irises included necrotic striated musculature, mononuclear cells, and granulocytes. Observed by electron microscopy, viral particles were embedded in the basement membrane of striated muscle cells, in membrane-bound vesicles in muscle cells, in proximity to pigmented epithelial cells, and in the lumen and basement membrane of endothelial cells of the iris. Necrosis of striated muscle cells occurred where large masses of viral particles were observed extracellularly and in vesicles in the cell. Particles budded from the cell membrane and the membrane surrounding vesicles.

It appeared that depigmentation of the irises of chickens with ocular leukosis was correlated with necrotizing changes of the striated muscle cells in the irises.

INTRODUCTION

Discoloration of the irises of chickens with ocular leukosis has been observed by several workers; the disease has been transmitted experimentally, with the production of neural and visceral lesions as well as ocular changes (7, 9, 10). In one experiment, the ocular lesion developed five days after the injection of 0.01 to 0.02 ml of a specific isolate into the anterior chamber of the eye (9). As a result, the pupil was dilated and elliptical or irregular in shape. The irises of birds with normal eyes were orange; the pupils were even and circular. The sciatic nerves of partially paralyzed chickens were edematous and yellow, as contrasted to the slender, white sciatic nerves of birds without ataxia. One of the birds with gray irises had white, nodular, tumorous masses in the liver. Another of the chickens having ocular lesions had a large ovarian tumor.

Histopathology. Small numbers of granulocytes and mononuclear cells consisting of lymphocytes and plasma cells were present in the irises of leukotic chickens. Striated muscle fibers in the irises were fragmented and necrotic (Fig. 1). The sciatic nerves of the four chickens with partial leg paralysis were edematous and yellow, as contrasted to the slender, white sciatic nerves of birds without ataxia. One of the birds with gray irises had white, nodular, tumorous masses in the liver. Another of the chickens having ocular lesions had a large ovarian tumor.

Materials and Methods

Five male and two female, 20-week-old chickens having bilateral lesions of ocular leukosis, were culled from a commercial breeder flock. Four of these birds had partial paralysis of the legs or wings. Eyes were secured from two normal chickens. At autopsy, small pieces of the sciatic nerves and irises of each chicken were fixed in 10% formalin, embedded in paraffin, cut at 5 μ, and stained with hematoxylin-eosin stain. An adjacent portion of iris was fixed in 2.5% buffered glutaraldehyde, postfixed in 1% OsO₄, and embedded in Araldite (6). No other tissues were examined with the electron microscope. Ultrathin sections on grids were stained with lead citrate (8) prior to being examined with a Philips EM200 electron microscope.

RESULTS

Gross Pathology. The irises of chickens that had ocular leukosis were gray. The pupils of these eyes were dilated and elliptical or irregular in shape. The irises of birds with normal eyes were orange; the pupils were even and circular. The sciatic nerves of partially paralyzed chickens were edematous and yellow, as contrasted to the slender, white sciatic nerves of birds without ataxia. One of the birds with gray irises had white, nodular, tumorous masses in the liver. Another of the chickens having ocular lesions had a large ovarian tumor.

Histopathology. Small numbers of granulocytes and mononuclear cells consisting of lymphocytes and plasma cells were present in the irises of leukotic chickens. Striated muscle fibers in the irises were fragmented and necrotic (Fig. 1). The sciatic nerves of partially paralyzed chickens were sparsely infiltrated by small lymphocytes. Tumors in the liver and ovary were composed of diffuse masses of cells with the characteristics of lymphocytes, plasma cells, and reticulum cells, many of the latter containing mitotic figures.

Electron Microscopy. The irises from normal chickens contained striated muscle cells, collagenous fibers, myelinated nerve fibers, and capillaries. Striated muscle cells containing lipid, glycogen, a nucleus, mitochondria, and sarcoplasmic reticulum had infoldings of the sarcolemma and were lined by a prominent basement membrane. The border of the iris toward the lens was lined by a layer of pigmented epithelial cells three to five cells thick. On their surfaces, these cells had elongated, twisted microvilli which intertwined with those of adjacent cells. The border of the iris toward the cornea was lined by a single layer of nonpigmented epithelial cells.

Although the iris of the leukotic eye contained collagenous fibers and myelinated nerve fibers similar to those observed in
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the normal eyes, degenerative changes were seen in striated muscle cells. Viral particles were usually embedded in basement membranes lining muscle cells (Fig. 2). For the most part, they accumulated in packets of three to twelve or more, especially in the invaginations of the cell membrane (Fig. 3). There was thickening and increased osmiophilia of the sarcolemma (Fig. 4) where the viral particle and cell membrane were in close apposition. It seems likely, as suggested by others (2, 4), that such thickening of the sarcolemma might be attributed to pinocytosis (Fig. 4 insert). Budding from the sarcolemma seemed to be involved in the separation of the virus from the cell cytoplasm (Fig. 5). Striated muscle cells also contained viral particles within membrane-bound vesicles. It appeared that such particles emerged as buds from the vesicular membranes (Fig. 6) and the peripheral cell membrane.

There appeared to be a correlation between severity of myopathy and extent of viral invasion of the muscle cell. Where there were few intravesicular particles, there was no disruption of myofibrils or alteration of their crossbanding (Fig. 7). Separation of myofibrils and disruption of banding (Fig. 8) occurred where extensive accumulations of extracellular particles and moderate numbers of intravesicular particles were observed. Necrosis and disappearance of myofibrils (Fig. 9) occurred in cells containing large masses of viral particles, both extracellularly and within vesicles. In addition, necrotic muscle cells contained myelinated figures, probably degenerated lipid droplets (Fig. 9). Mitochondria were altered in cells that were heavily laden with virus; such organelles were swollen, misshapen, and contained fragmented cristae (Fig. 10).

Viral particles were not observed within membrane-bound spaces in pigmented epithelial cells, but sizeable clusters of virus were trapped in the invaginations of the plasmalemma formed by branching microvilli on the surfaces of these pigmented cells (Fig. 11). As a result, viral particles could be mistakenly interpreted as being present within membrane-bound vesicles in the cells.

Viral particles were also seen in the capillaries of the iris. Particles were observed not only in lumens of these blood vessels, but they were also embedded in the basement membrane of endothelial cells and in pericytes of the capillaries (Fig. 12).

Viral particles had a uniform ultrastructural appearance, regardless of location. They varied from 110 to 130 μm in diameter, had an inner ring and an outer membrane, and had a central nucleoid which varied in size from 40 to 46 μm. The inner ring was separated from the nucleoid, and the outer membrane had surface projections (Fig. 13).

Viral particles were not seen in the nucleus of muscle cells. A few plasma cells, granulocytes, and lymphocytes were present in the vicinity of necrotic muscle cells.

DISCUSSION

Irises having lesions typical of ocular leukosis were examined in the present work. Previous investigators have reported that ocular leukosis, “gray eye,” resulted from infiltration of the iris with lymphocytes, plasma cells, and granulocytes, resulting in a grayish, deformed, and paralyzed iris (1, 3). These cells were scarce in the ocular lesions studied by light and electron microscopy in the investigation reported herein. Instead, the significant observation was the presence of a multitude of viral particles in membrane-bound vesicles and around striated muscle cells, in the close vicinity of pigmented epithelial cells, and in the lumen, basement membrane, and pericytes of capillaries. Muscle cells heavily infected with virus, both intravesicular and extracellular, were necrotic. Thus, it was concluded that necrotizing changes of the striated muscle were correlated with color change of the iris, as well as notching or rupture. Comparisons with other works could not be made because there do not appear to be any previous reports of electron microscopic studies of the irises of leukotic chickens.

The viral particles observed in irises of leukotic eyes appeared to be similar to those of the leukosis-sarcoma group of viruses which emerge as buds from the cell membrane (5, 12, 13) and exhibit pinocytosis (4). The agent in the iris was ultrastructurally similar to that described in the peristeous of chickens with experimental osteopetrosis (11).

ACKNOWLEDGMENTS

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REFERENCES

Fig. 1. Striated musculature (M) is fragmented in the iris of a bird having ocular leukosis. The cells (arrows) in that area are lymphocytes, plasma cells, and granulocytes. H & E, × 125.

Fig. 2. Several packets of viral particles (arrows) are embedded in the basement membrane of a striated muscle cell. × 15,000.

Fig. 3. Viral particles (V) are present in invaginations of the sarcolemma of muscle cells. × 25,000.

Fig. 4. There is thickening and increased osmiophilia of the muscle cell sarcolemma (arrows) where it is in close apposition with viral particles (V). × 30,000. Insert. The thickened sarcolemma (arrow) has almost surrounded a viral particle. × 45,000.

Fig. 5. Sequence of increasing separation of virus from the muscle cell sarcolemma by the process of budding (arrow) as shown in steps a through d. × 100,000.

Fig. 6. Virus particles seem to emerge as buds from the sarcolemma (arrow) of a striated muscle cell and from the membrane lining intracellular vesicles (V). × 55,000.

Fig. 7. Disruption of myofibrils does not occur if there are only a few intravesicular particles present (arrows). The delicate, granular material is glycogen (G). There are many extracellular particles (V) embedded in the basement membrane of the muscle cell. × 20,000.

Fig. 8. Disruption of myofibrils and bandings (B) occurs when there are numerous extracellular particles (V) and a moderate number of intravesicular particles (arrows) in a muscle cell. × 20,000.

Fig. 9. Necrosis of the muscle cell and disappearance of fibrils (F) occur when there are large masses of virus, both extracellular (V) and intravesicular (arrows). × 18,000. Insert. A myelinated figure in the cytoplasm (M), and virus (V) in the basement membrane. × 12,000.

Fig. 10. Lipid (L) in cytoplasm of a muscle cell with extracellular virus (V) has a normal appearance; mitochondria (M) are swollen and the cristae fragmented. × 18,000.

Fig. 11. In pigmented epithelial cells, viral particles (V) are present in invagination of the plasmalemma, formed by branching microvilli (arrow) on the surface of the cells. × 30,000. Insert. The structure of branching microvilli (arrow) on the surface of pigmented epithelial cells can be observed. × 12,000.

Fig. 12. Viral particles (V) are free in the lumen and are embedded in the basement membrane (arrows) of endothelial cells of a capillary. × 12,000.

Fig. 13. Viral particles embedded in the basement membrane of a muscle cell have inner (I) and outer (O) membranes and a nucleoid (N). × 100,000.
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