Electron Microscopical Observations of Psittacine Beak and Feather Disease in an Umbrella Cockatoo (Cacatua alba)

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ABSTRACT. Psittacine beak and feather disease (PBFD) was diagnosed in an umbrella cockatoo (Cacatua alba) with severe feather dystrophy and loss. Electron microscopically, the intranuclear and intracytoplasmic inclusion bodies observed by light microscopy were composed of viral particles forming paracrystalline arrays, whorls, semicircles or concentric circles. Recovered viral particles from the skin and feather follicle tracts were icosahedral and 15 to 20 nm in diameter.—KEY WORDS: cockatoo, electron microscopy, psittacine beak and feather disease.

Psittacine beak and feather disease (PBFD) was first described in South Pacific psittacine birds in the mid 1970s [4]. This is a major disease in many species of psittacine bird [5]. The cause of the disease is an icosahedral and nonenveloped virus 14 to 17 nm in diameter [4]. It has been suggested that PBFD virus, chicken anemia virus and nonpathogenic porcine circovirus are in the same family called Circoviridae [1, 7]. Symmetric feather dystrophy and loss with occasional beak overgrowth are characteristic lesions [3, 4]. Various findings in the pathogenesis, pathology and diagnosis of PBFD have been accumulated by Australian and American researchers [2–4, 6, 8–16, 18], but our previous report [17] is the only one in Japan concerning PBFD in psittacine birds. This article reports the electron microscopical observations in a naturally PBFD infected umbrella cockatoo in Japan.

A 4–5 year-old bird (Umbrella cockatoo, Cacatua alba) with severe feather dystrophy and loss and mild beak overgrowth died and was donated to our laboratories for microbiological and pathological examination. A detailed necropsy was performed on the bird and tissues were fixed in 10% phosphate buffered formalin. Numerous areas of skin from the neck and legs and other systemic organs were processed routinely in paraffin, sectioned at 4 μm, and stained with hematoxylin and eosin (HE). For electron microscopy, formalin-fixed tissues (skin neck, liver and spleen) from the bird were post-fixed in 1% osmium tetroxide and embedded in epon. Ultra-thin sections from selected areas were stained with uranyl acetate and lead citrate, and observed with an 1200-EX electron microscope (JEOL). Recovery of the PBFD virus was done from feather follicle tracts of the diseased bird by a published method [16]. Briefly, frozen feather follicle tracts were thawed, minced and homogenized. After centrifugation at 1,000 × g for 10 min, the supernatant was layered onto a 45% sucrose cushion in Tris buffer and centrifuged at 140,000 × g for 2 hr at 4°C. The crude viral pellets were resuspended in Tris buffer, adjusted to 1.40 g/cc with cesium chloride and centrifuged to equilibrium at 270,000 × g for 16 hr at 20°C. Fractions from cesium chloride equilibrium gradients were collected from the bottom of tubes. Cesium chloride-purified virus suspension was negatively stained.

The feathers were yellowish, short, clubbed, deformed or curled, with thickened or retained feather sheaths, hemorrhage within the pulp cavity, fractures of the proximal rachis and failure of developing feathers to exsheath, mild overgrowth of beak, and dehydration of skin. At necropsy the bird showed signs of hepatomegaly, discolored kidneys and atrophy of the spleen, thymus and bursa of Fabricius. Microscopically, feather lesions were mild to moderate hyperkeratosis of feather sheaths and scattered mild to severe epithelial cell necrosis, particularly in the basal and intermediate layers of the feather epithelium. The sheath of those feathers in which the feather was retained, were thickened and often contained macrophages. Changes within the feather pulp generally varied, and often the pulp was edematous and infiltrated by heterophils and macrophages. Intracytoplasmic and intranuclear inclusion bodies were observed within the feather epithelium and feather follicle epithelium. Macrophages with conspicuous multiple globular intracytoplasmic inclusion bodies of various sizes and numbers were sometimes present in the epidermis, sheath and pulp (Fig. 1). Most of these inclusion bodies were basophilic. Eosinophilic intracytoplasmic inclusion bodies were observed in the Kupffer cells and splenic macrophages in the white pulp. Atrophy of lymphoid tissue and focal aggregates of necrotic cells were observed in the thymus and bursa of Fabricius. Electron microscopic examination of feathers revealed necrosis of epidermal cells and engulfment of necrotic debris by macrophages. Electron microscopically, the basophilic intranuclear and intracytoplasmic inclusion bodies observed in the feather epithelium and feather follicle epithelium by light microscopy were composed of viral particles 15 to 20 nm in diameter, that formed paracrystalline arrays, whorls, semicircles, concentric circles and other configurations (Fig. 2). On the other hand, eosinophilic intracytoplasmic inclusion bodies observed in the Kupffer cells and splenic
macrophages were composed of similar viral particles forming paracrystalline arrays. Ultrastructural analysis of negatively-stained virus preparations revealed icosahedral, nonenveloped viral particles 15 to 20 nm in diameter (Fig. 3).

In the present study we first succeeded in confirming PBFD viral particles from a diseased bird in Japan. The viral particles 15 to 20 nm in diameter purified from the skin including feather follicles of the affected bird were confirmed to be those of PBFD virus by their ultrastructural features. The viral inclusion bodies seen in this study were also observed in the cells described in other reports [1, 3–6, 16–18], such as macrophages in the feather epithelium and feather pulp, feather epithelial cells, feather follicular epithelial cells, Kupffer cells and splenic macrophages. Electron microscopically, intranuclear and intracytoplasmic inclusion bodies were seen to consist of viral particles 15 to 20 nm in diameter forming paracrystalline arrays, whorls, semicircles, concentric circles and other configurations. The size and arrangement of these viral particles were similar to those found in psittacine birds reported in Australia and America [4, 18]. Light and electron microscopical evidence suggests that the cells targeted by PBFD virus may be epithelial cells in the feathers and feather follicles and macrophages in various tissues. PBFD has been associated with acute infections in young psittacine birds, as well as with chronic infections in older psittacine birds [14]. The present case is considered to be one of chronic PBFD because of the age and clinical features of the diseased bird. In addition, chronic PBFD in birds is frequently of an immunosuppressive nature [15].

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Fig. 1. Macrophages with conspicuous multiple globular intracytoplasmic inclusion bodies are seen in the feather pulp. Skin. HE stain, × 600.

Fig. 2. Electron micrograph of cytoplasmic inclusion bodies in a macrophage. Viral particles are in paracrystalline arrays and concentric circles. × 12,000. At a higher magnification (inset). × 40,000.
Fig. 3. Electron micrograph of viral particles recovered from the diseased cutaneous tissues. Negatively stained with uranium acetate. Bar=100 nm.

REFERENCES