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DISEASE IN WILDLIFE OR EXOTIC SPECIES

Cytomegalic Inclusion Disease caused by Cytomegalovirus Infection in the Salivary Glands of an African Hedgehog (*Atelerix arbiventris*)

K. Koyama[*,†], N. Horiuchi[*], H. Hasegawa[‡], H. Katano[‡], E. Oohashi[§], K. Watanabe[*] and Y. Kobayashi[*]

** Laboratory of Veterinary Pathology, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, † The United Graduate School of Veterinary Sciences, Gifu University, Gifu, ‡ Department of Pathology, National Institute of Infectious Diseases, Shinjuku and § Akashiya Animal Hospital, Obihiro, Japan*

Summary

Cytomegalic inclusion disease (CID) in the salivary gland of African hedgehogs (*Atelerix arbiventris*) has been reported before, and is suspected to reflect a cytomegalovirus infection. However, a recent ultrastructural study reported that African hedgehog CID reflected oncocytic metaplasia, mimicking a cytomegalovirus infection. We examined the submandibular and sublingual salivary glands of a 1-year-old male African hedgehog. Histologically, there were multiple foci composed of cytomegalic cells with intranuclear inclusion bodies. Ultrastructurally, viral particles (109–118 nm in diameter) were observed in the nuclei of the cytomegalic cells. There were numerous vesicles containing various numbers of enveloped viruses in the cytoplasm. We also attempted to detect viral DNA fragments by degenerate polymerase chain reaction and obtained amplicons of a predicted size. Phylogenetic analysis indicated that the virus is a betaherpesvirus, comparatively related to human and rodent cytomegaloviruses. The present study suggested that African hedgehog CIDs also include those caused by the cytomegalovirus.

Keywords: African hedgehog; cytomegalic inclusion disease; cytomegalovirus; salivary gland

Cytomegalic inclusion disease (CID) in the salivary glands is common in animals that belong to the order Eulipotyphla (former Insectivore), such as shrews (*Suncus murinus*, *Crocidura dsinezumi* and *Sorex unguiculatus*) and moles (*Mogera kobeae* and *Urotrichus talpoides*) (Mineda, 1981; Cosgrove, 1986). In the salivary glands of these species, herpesvirus-like particles have been observed at high rates by ultrastructural examination (Mineda, 1981). The African hedgehog (*Atelerix arbiventris*) is classified in this order. In addition, CID in the salivary glands of the African hedgehog has also been reported for over 40 years, and was suspected to reflect a cytomegalovirus infection (Karstad, 1975). However, Brunnert *et al.* (1991) reported that the responsible virus had not been detected by ultrastructural examination in all three cases of African hedgehog CID. Moreover, there were numerous enlarged mitochondria in the cytoplasm of the cytomegalic epithelial cells, and they therefore concluded that African hedgehog CID reflected oncocytic metaplasia (Brunnert *et al.*, 1991). The present report describes CID lesions caused by cytomegalovirus in the salivary glands of an African hedgehog, in a case diagnosed histopathologically as 'wobbly hedgehog syndrome' and adenocarcinoma of the pancreas.

A 1-year-old male African hedgehog, raised as a companion animal, presented with neurological signs. The hedgehog died 40 days after first examination

Correspondence to: Y. Kobayashi (e-mail: kyoshi@obihiro.ac.jp).

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and pathological examination was performed in our laboratory. At necropsy examination, the salivary glands showed no gross lesions.

Tissue samples of the submandibular and sublingual salivary glands were fixed in 15% neutral buffered formalin, processed routinely and embedded in paraffin wax. Sections (3 μm) were stained with haematoxylin and eosin (HE). Selected sections were subjected to the periodic acid—Schiff (PAS) reaction and immunohistochemistry (IHC) using the following primary antibodies: monoclonal anti-mitochondria (diluted 1 in 100; NeoMarkers, Fremont, California, USA) and anti-human cytomegalovirus (HCMV), according to a previous report (Maeda *et al.*, 1994). For electron microscopy, the formalin-fixed submandibular gland was post-fixed in 1% phosphate-buffered osmium tetroxide (pH 7.4) and embedded in resin. Ultrathin sections were stained with uranyl acetate and lead citrate. DNA from the formalin-fixed, paraffin wax-embedded submandibular gland was isolated using Nucleospin Tissue™ (Macherey-Nagel, Düren, Germany). Degenerate polymerase chain reaction (PCR) targeting the highly conserved glycoprotein B (gB) gene of betaherpesviruses was performed according to a previous report with the following slightly modified degenerate primer set: CM-gB1 forward (5′ TTC AAG GAA CTC AGY AAR ATN AAY CC 3′), CM-gB1 reverse (5′ CGT TGT CCT CNC CAN RYT GNC C 3′), CM-gB2 forward (5′ CGA AAC ATC ATG GAN KCN TGG TG 3′), CM-gB2 reverse (5′ CGT TGT TCT CNC CAN RYT G 3′), CM-gB3 forward (5′ TTG AGA AAC ATT TTN GAN GCN TGG TG 3′), CM-gB3 reverse (5′ TCT AAA CGT CCC AAG AAG NAT YTC RTT 3′) (Ehlers *et al.*, 2007). Direct sequencing of the purified amplicons was performed. The sequences were analyzed using Sequence Scanner Software Version 2.0 (Applied Biosystems, Foster City, California, USA) and aligned using the Clustal W algorithm. The sequence data were compared with published sequences in the GenBank databases using the basic local alignment search tool (BLAST).

Histologically, multiple foci composed of cytomegalic cells were observed in the submandibular and sublingual salivary glands (Fig. 1). The cytomegalic cells had pale eosinophilic to amphophilic foamy cytoplasm, and enlarged nuclei that had thickened nuclear membranes and contained eosinophilic to amphophilic intranuclear inclusion bodies. Some nuclei also contained obvious nucleoli. Cytomegalic cells were also found within normal acinar and ductal epithelium. Acinar cells of other areas were mildly atrophic. There was slight mononuclear inflammatory cell infiltration around the foci and in the interacinar interstitium. The foci of cytomegalic cells were composed of several small foci separated by PAS-positive basement membranes.

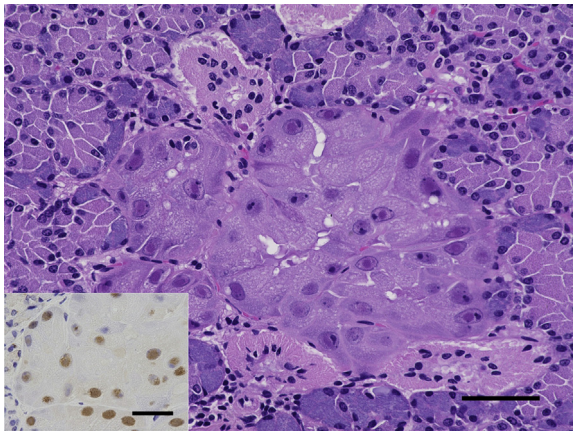

Fig. 1. Focus of cytomegalic cells with foamy cytoplasm and enlarged nuclei containing eosinophilic to amphophilic intranuclear inclusion bodies. HE. Bar, 50 μm. Inset: Positive labelling is observed of intranuclear inclusion bodies or diffusely in the nuclei. IHC. Bar, 50 μm.

Immunohistochemical labelling using an anti-HCMV antibody revealed positive reactions in intranuclear inclusion bodies or diffusely in the nuclei (Fig. 1, inset). The amount of granular mitochondrial immunoreactivity was different for each cytomegalic cell. Mitochondria filling the cytoplasm were not observed. Ultrastructurally, intranuclear inclusion bodies consisted of dense reticular or filamentary structures and surrounded numerous viral particles (Fig. 2). Viral capsids were 109—118 nm in diameter. Many of the capsids contained dense or beaded cores and others were empty. In the cytoplasm of the cytomegalic cells, there were many vesicles containing

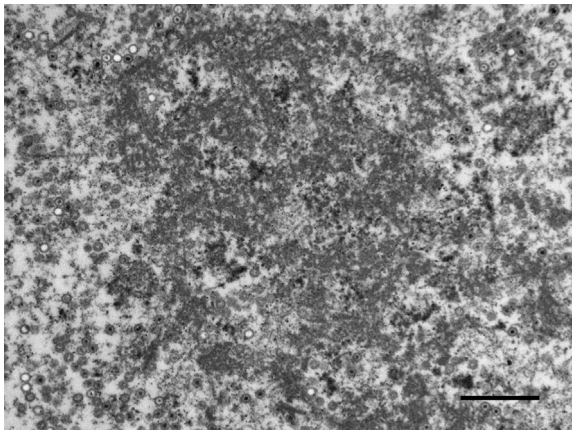

Fig. 2. Electron micrograph of an enlarged nucleus. The intranuclear inclusion body consists of dense reticular or filamentary structures and is surrounded by numerous viral particles. TEM. Bar, 1,000 nm.