Case Report

Granulomatous Nephritis Consistent with Malakoplakia in a Cynomolgus Monkey

Yoshikazu Taketa[1*], Akira Inomata[1], Jiro Sonoda[1], Kazuhiro Hayakawa[2], Kyoko Nakano-Ito[1], Etsuko Ohta[1], Yuki Seki[1], Aya Goto[1], and Satoru Hosokawa[1]

[1] Tsukuba Drug Safety, Global Drug Safety, Biopharmaceutical Assessments Core Function Unit, Eisai Product Creation Systems, Eisai Co., Ltd., 5-1-3 Tokodai, Tsukuba, Ibaraki 300-2635, Japan
[2] Preclinical Safety Research Laboratories, Kawashima Division, Sunplanet Co., Ltd., 1 Kawashimatakehaya-machi, Kagamigahara, Gifu
501-6195, Japan

Abstract: Malakoplakia is a rare form of chronic granulomatous inflammation in mammals, and usually affects the urinary tract in humans. In this report, we present a case of granulomatous nephritis consistent with malakoplakia in a 4-year-old male cynomolgus monkey. Gross examination showed that the kidney was markedly enlarged and adhered to the surrounding organs. Histology showed that there was diffuse interstitial infiltration of histiocytes with abundant foamy eosinophilic cytoplasm resembling von Hansemann cells, PAS-positive granular cytoplasm and occasional PAS- and iron-positive intracellular small inclusion bodies. Electron microscopy showed that these histiocytes contained abundant lysosomes and phagolysosomes but no obvious Michaelis-Gutmann bodies. Based on these findings, a diagnosis of granulomatous nephritis consistent with early malakoplakia was made. This is the first report in a monkey of a renal lesion consistent with malakoplakia. (DOI: 10.1293/tox.2013-0024; J Toxicol Pathol 2013; 26: 419–422)

Key words: granulomatous nephritis, Malakoplakia, cynomolgus monkey, kidney

Malakoplakia is a rare granulomatous inflammatory disorder primarily affecting the urinary bladder in humans[1]. It also affects other organs, most notably the kidneys[2–4]. Histologically, the lesion is characterized by a diffuse sheet of histiocytic cells, known as ''von Hansemann cells,'' which are filled with small granules that stain positive with the periodic acid Schiff (PAS) reaction and are resistant to diastase treatment[5]. PAS-, calcium- and iron-positive and laminated inclusion bodies are found within and between histiocytes and are known as ''Michaelis-Gutmann'' (MG) bodies. Reports of naturally occurring malakoplakia in domestic and companion animals are limited to pigs[6,7] and a kitten[8].

A 4-year-old male cynomolgus monkey imported from China (Guangxi Grandforest Scientific Primate Co., Ltd., Guangxi, China) in the low-dose group of a 13-week toxicity study was euthanized at the end of the dosing period. This animal was individually housed in stainless steel cage (D680 × W620 × H770 mm) in an animal room maintained under controlled conditions (temperature, 26 ± 3°C; relative humidity, 55 ± 20%), was given approximately 108 g/day of diet for monkeys (HF Primate 5K91 12G 5K9J, Purina Mills, LLC) in the afternoon each day and was allowed free access to tap water. At necropsy, the animal was euthanized by exsanguination under sodium pentobarbital anesthesia from the cephalic vein. During the dosing period, this animal lost about 1 kg of body weight (3.73 kg pre-dose; 2.81 kg at 13 weeks). Clinical pathological examinations revealed elevated blood urea nitrogen (23.0 mg/dL pre-dose; 53.1 mg/dL at 13 weeks), anemia [decreased red blood cells (6.11 10^6/mm^3 pre-dose; 3.64 10^6/mm^3 at 13 weeks), hematocrit (47.0% pre-dose; 25.5% at 13 weeks) and hemoglobin (14.7 g/dL pre-dose; 7.8 g/dL at 13 weeks)], neutrophilia (8.77 10^3/mm^3 pre-dose; 28.12 10^3/mm^3 at 13 weeks) and electrolyte imbalance [increased potassium (4.0 mEq/L pre-dose; 5.8 mEq/L at 13 weeks) and decreased sodium (149 mEq/L pre-dose; 139 mEq/L at 13 weeks) and calcium (10.2 mg/dL pre-dose; 8.3 mg/dL at 13 weeks)]. There was no change in the results of urinalyses performed by macroscopic observation or using test papers, an automatic urine analyzer or sediment microscopy. At necropsy, the right kidney was enlarged and adhered to the adrenal, liver, large intestines and abdominal wall. On the cut surface, a poorly-demarcated white solid area was located in the renal cortex and spread into the surrounding parenchyma. No other animals in the study had similar findings. Tissues were fixed in 10% neutral buffered formalin and embedded in paraffin, and sec-

tions were stained with hematoxylin and eosin (H&E), PAS, Berlin blue, von Kossa, Gram and Ziehl-Neelsen stains. Additionally, immunohistochemical staining for CD204 (SRA-E5; Transgenic, Kumamoto, Japan), Iba1 (019-19741; Wako Pure Chemical Industries, Osaka, Japan) and lysozymes (422491; Nichirei Bioscience, Tokyo, Japan) was performed to confirm the presence of histiocytes. For ultrastructural observation, the formalin-fixed tissue was postfixed in buffered glutaraldehyde, followed by osmium tetroxide, and embedded in plastic resin. Ultrathin sections were stained with lead-uranyl acetate and examined with a Hitachi H-7650 transmission electron microscope.

Histology showed that the white solid area in the renal cortex was macroscopically consistent with diffuse dense sheets of large foamy histiocytes admixed with lymphocytes and neutrophils spread in the renal interstitium. Inflammation was mainly in the cortex and the outer medulla (Fig. 1) and was accompanied by capsular fibrosis, which also involved the surfaces of grossly adhered adjacent organs and tissues near the lesion. Focal necrosis and microabscess formation were occasionally present in the renal lesion. Degenerative or regenerative renal tubules were observed, but glomeruli were mostly intact within the affected area (Fig. 1). Histiocytes contained abundant fine eosinophilic PAS-positive cytoplasmic granules (Fig. 2a and b). Cytoplasmic inclusion bodies were positive for PAS and iron (Berlin blue) (Fig. 2b and c) and negative for calcium (von Kossa). Immunohistochemistry showed that these cells were positive for macrophage markers CD204 (Fig. 2d), Iba1 and lysozymes. Electron microscopy showed that histiocytes had a ruffled membrane with small membranous projections and contained abundant lysosomes and phagolysosomes (Fig. 3). Typical MG bodies, which are concentrically laminated with an irregular outer surface and less electron-dense core with calcium deposits, were not present. Gram, PAS and Ziehl-Neelsen staining did not reveal any microorganisms. The urinary bladder and contralateral kidney were normal, and there were no granulomatous lesions in any other organs.

Based on the tissue distribution and microscopic morphology, a diagnosis of granulomatous nephritis consistent with malakoplakia was made. A diagnosis of malakoplakia is generally made based on the presence of large polygonal or round-shaped macrophages with foamy eosinophilic cytoplasm accompanied by MG bodies, which are believed to be associated with inadequate processing of phagocytosed bacteria[1,9]. However, MG bodies are found only in fully developed malakoplakia, and may be sporadic or inconspicuous in the early stages[9,10]. Taken together, although the present case lacked typical MG bodies with calcium deposits, the extensive infiltration of interstitial macrophages containing PAS- and iron-positive intracytoplasmic inclusion bodies consistent with von Hansemann cells supports diagnosis of early-stage malakoplakia.

Differential diagnoses in this case included xanthogranulomatous pyelonephritis and renal cell carcinoma (RCC). Xanthogranulomatous pyelonephritis is a histologic lesion similar to malakoplakia; but the presence of PAS-positive cytoplasmic granules and MG bodies differentiate malakoplakia from xanthogranulomatous pyelonephritis in humans[10,11]. Large-sized foamy cells may be reminiscent of RCC; however, a variety of inflammatory cell infiltration and a series of results indicating the macrophage-derived foamy cells clearly distinguish this lesion from RCC.

In humans, malakoplakia in the urinary tract is most commonly observed in the urinary bladder, particularly that of middle-aged women[12]. Compared with the disease in humans, the present case was different in that the occurrence was in a male animal and that the lesion was observed in the unilateral kidney without any other changes in the urinary tract including the urinary bladder.

The pathogenesis of malakoplakia remains obscure, but it is currently proposed that it is caused by a compromise of the structure and function of phagolysosomes due to defective assembly of macrophage microtubules[5]. Degradation of phagocytosed materials is therefore impaired, and these materials accumulate in the cytoplasm as PAS-positive granules. They also act as a nidus for the lamellar deposition of calcium and iron to form MG bodies. Gram-negative bacteria are often associated with malakoplakia, with *E. coli* found in approximately 70 to 90% of cases[9,13]. Because of difficulties with histological approaches including special Gram, PAS and Ziehl-Neelsen stains in detecting the microorganisms, site-specifically amplified PCR analysis is said to be useful for the determination of pathogens in malakoplakia[9]. Malakoplakia has been experimentally induced in pigs by subcutaneous injection of *Rhodococcus equi*, and can be induced in rats by intrarenal and intratesticular injection of *E. coli* endotoxin-antigen complex[14,15]. These findings support an association with bacterial involvement in the formation of malakoplakia. Although there were no obvious changes in urinalysis or histologic changes in the urinary bladder, the urinary tract infection was considered to be most likely caused by the location of the lesion.

About 20% of patients with malakoplakia in humans

Fig. 1. Low-magnification image of the renal lesion. The lesion consists of diffuse interstitial infiltration of large and eosinophilic cells and inflammatory cells, occasional degenerative or regenerative renal tubules and intact glomeruli (H&E staining). Bar = 100 μm.

Fig. 2. H&E (a), PAS (b) and Berlin blue (c) stainings and immunohistochemistry for CD204 (d) at highmagnification. H&E staining shows the large-sized foamy cells with abundant fine eosinophilic cytoplasmic granules and eosinophilic inclusion bodies (arrowheads) (a). PAS-positive granular cytoplasm and PAS-positive round to irregularly shaped inclusion bodies (arrowheads) (b). Inclusion bodies are also positive for iron staining (Berlin blue, arrowheads) (c). The eosinophilic foamy cells are positive for CD204 immunostaining (d). Bars = 20 μm.

Fig. 3. Ultrastructure of the infiltrated histiocytes; the cells have abundant lysosomes and phagolysosomes in the cytoplasm (arrowheads). Bar = 5 μm.
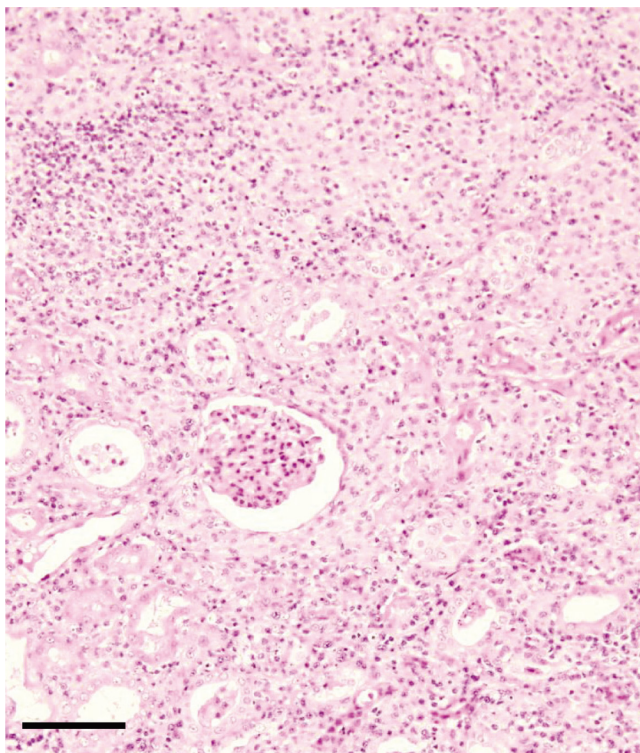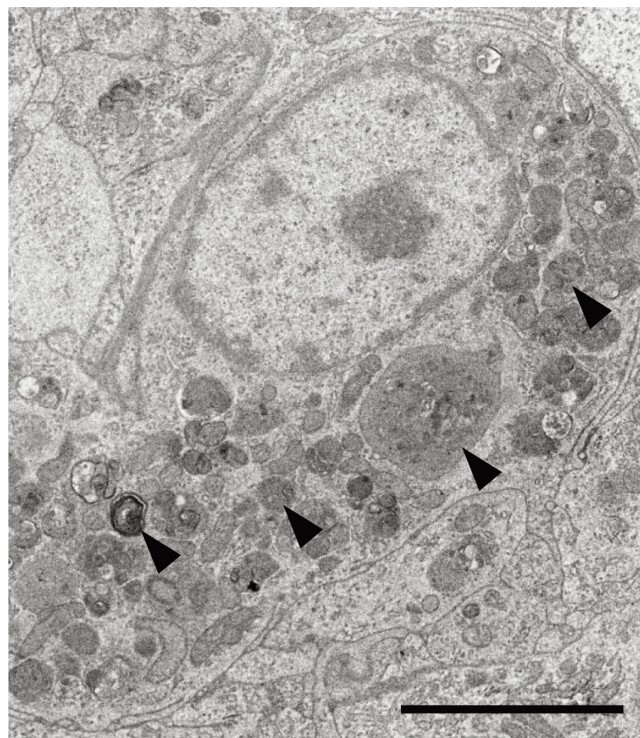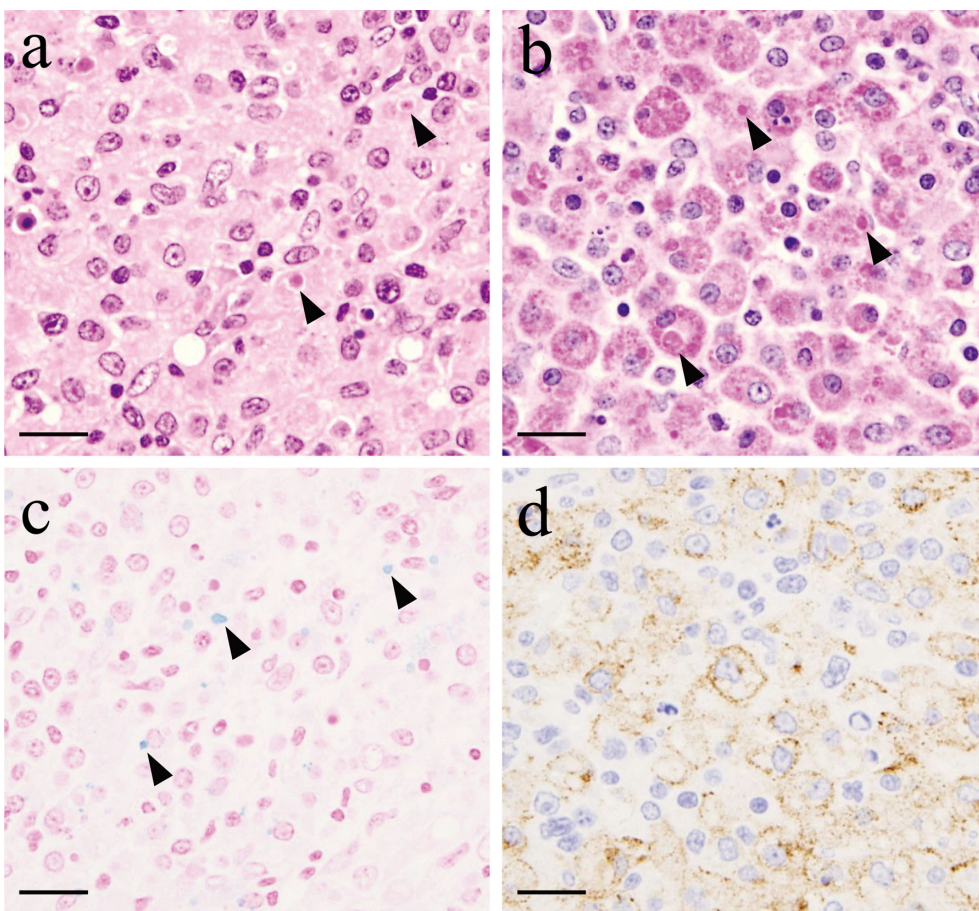
Fig. 1.

Fig. 3.

Fig. 2.

have some form of immunosuppression, including autoimmune diseases and after the surgery of kidney transplant requiring steroids or azathioprine[16]. Since the present case was accompanied by lymphoid depletion in the submaxillary and mesenteric lymph nodes, spleen and thymus, these conditions may be involved in the cause of this lesion. Taken together, multiple mechanisms including bacterial infection, macrophage dysfunction and immune deficiency are considered to be involved in the development of malakoplakia.

In conclusion, the present renal granulomatous lesion had characteristics consistent with malakoplakia. This is the first report of malakoplakia in a monkey.

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