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National Institutes of Health
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CASE I – MK05-4177 (AFIP 2992272)

Signalment: Adult, intact, male, rhesus macaque, *Macaca mulatta*

History: This monkey was challenged with simian immunodeficiency virus (SIV) approximately 4.5 years ago and treated with a regimen of antiretroviral therapy. The animal had a 4 month history of dry skin and a recurring nail infection, both of which responded to medical treatment, as well as a 4 month history of poor appetite. Lesions on his back and in his mouth were observed 11 days prior to necropsy.

Gross Pathology: On necropsy the monkey was in poor nutritional condition with numerous bony protuberances and scant adipose stores. Multifocal, varying sized proliferative lesions with central areas of ulceration were observed randomly scattered about the oral and gingival mucosa. There were two ulcerated areas on the skin of the left lateral thoracic region, one measuring 1.5 cm and the other measuring 1 cm in diameter. The entire left lung field was mottled dark purple, dark grayish red and red and palpated meaty. The left caudal lung lobe had pleural adhesions. The right lung field was mottled reddish gray, red, and pink, and a small amount of crepitus was noted upon palpation. Lesions were not observed in any other organs.

Laboratory Results: PCR on mesenteric LN was negative for *Mycobacterium* spp.

Histopathologic Description: The samples submitted were sections of lip with a mucosal side and a haired skin side, although not all slides may have had haired skin present. The lesions of interest were in the oral mucosa; however, some sections showed involvement of the haired skin as well.

Involving the oral mucosa was a focally extensive area of ulceration that peripherally had epithelial hyperplasia. Associated with the ulcerated areas were inflammatory cells. The inflammatory cell population was largely comprised of neutrophils and macrophages admixed with cellular debris. Multiple epithelial cells at the margin had slightly expanded and vacuolated cytoplasm. Numerous other cells, individually or as syncytia, were expanded and contained amphophilic to basophilic intranuclear inclusion bodies.

Contributor's Morphologic Diagnosis: Oral cavity: Stomatitis, ulcerative, acute to subacute, multifocal, moderate to severe with intranuclear inclusion bodies.

Contributor's Comment: The most likely cause of the oral lesions in this case is Herpes Simplex Virus (HSV), which is common in HIV infected patients. The most common site of infection is the mucocutaneous junction of the lip, although lesions can be found elsewhere on the oral mucosa, conjunctiva, skin, esophagus, and external genitalia (7, 11, 13). Histologically, HSV infections appear with necrosis, ballooning degeneration, multinucleated giant cells, and intranuclear inclusion bodies (7).

There are three main subfamilies within Herpesviridae that infect monkeys: alpha-herpesviruses (simplex virus, simian varicella, B virus), beta-herpesviruses (cytomegaloviruses), and gamma-herpesviruses (lymphoproliferative viruses) (5). Most of the herpesviruses that infect nonhuman primates belong to the alpha-herpesviruses, which generally cause mild disease in the natural host but can cause life-threatening disease in unnatural hosts. The lymphoproliferative herpesviruses, such as Epstein-Barr virus, rhesus lymphocryptovirus, and rhadinovirus are generally associated more with lymphoproliferative disorders as opposed to ulcerative dermatitis (2, 5, 9). Herpes B, an alpha-herpesvirus, is a common virus of rhesus macaques that can cause lesions on the dorsal surface of the tongue and at the mucocutaneous junction; however, the gross lesions are generally only associated with an acute primary infection and are rarely noted in chronically infected animals (6). Another alpha-herpesvirus, simian varicella (Medical Lake Macaque virus), causes a chicken pox-like disease that forms a papule to a vesicle in gross appearance (4, 12, 14). Histologically, infected cells have enlarged nuclei with discrete intranuclear inclusions, and so should be considered as the possible etiologic agent in this case; however, there is not much evidence in the literature that rhesus macaques get infected. The most common herpesvirus associated with SIV infections of rhesus monkeys is the rhesus monkey cytomegalovirus (CMV) which causes a systemic infection causing areas of necrosis in multiple organs and producing large intranuclear basophilic inclusions (11, 13).

Monkeys that have been infected with SIV can display a variety of clinical signs and lesions, largely dependent on the duration and stage of infection. This particular monkey had been infected for three and a half years by the time of death; therefore the gross findings were reflective of the chronicity of the infection. Lymphoid tissues are generally the principal target organs for SIV, and with prolonged SIV infections we expect to see depletion of follicles, paracortices, and periarteriolar lymphoid sheaths (3, 8, 10). In this animal, moderate to marked lymphoid depletion was noted in all lymph nodes as well as the spleen. In addition, this animal also had a markedly involuted thymus, which is a consistent sign among monkeys with late stage SIV infections regardless of age (8).

The hallmark sign of AIDS in either humans or macaques is severe immunosuppression resulting from an impairment primarily of cell-mediated immunity, especially CD4+ T cells (3). Immunosuppression then leads to secondary opportunistic infections. In this animal, the ulcerative lesions on the oral mucosa are associated with a secondary, herpesvirus infection.

In SIV infected animals, opportunistic infections are the hallmark of the development of AIDS, and in many cases some of the first signs are cutaneous lesions (11, 13). There are several etiologies that are commonly associated with oral and cutaneous lesions. Of the bacterial infections that complicate SIV infection and simian AIDS, *Staphylococcus aureus* is the most common (11, 13). These lesions are characterized by folliculitis, abscesses, and cellulitis. Mycobacterial disease, usually caused by *Mycobacterium tuberculosis* and *M. avium intracellulare*, is also known to complicate SIV or HIV infection (11, 13). The usual lesions are a tuberculosis chancre that begins as a painless nodule and forms an ulcer; however, skin lesions with tuberculosis are rarely encountered in human practice (11).

There are several secondary fungal infections that can cause disease in HIV/SIV infections. The most common fungal infection associated with AIDS is *Candida*, the cause of thrush (oral candidiasis) (11, 13). *Candida* is a part of the normal flora of the oropharynx and gastrointestinal tract, and so is a common source of secondary infections. The lesions appear as white plaques, usually on the tongue or oral mucosa, that when scraped off leave a bleeding ulcer behind. Histologically, pseudohyphae and spores are evident in the stratum corneum (13). Cryptococcosis is another fungal infection that is transmitted by inhaling spores from the environment, and disseminates readily in immunocompromised patients (13). The lesions associated with cryptococcosis are papules, nodules, pustules, and ulcerations that mainly involve the head and neck. Lastly, coccidioidomycosis can cause disseminated cutaneous involvement in AIDS patients (13). In addition, *Pneumocystis carinii* has been commonly identified as an opportunistic pathogen (13).

Parasitic infections must also be included when considering secondary infections associated with SIV. The most common parasitic infection of patients with HIV is *Sarcoptes scabiei*, which generally cause hyperkeratosis and plaque formation distributed diffusely (11, 13).

AFIP Diagnosis: Lip: Cheilitis, ulcerative, multifocal, subacute, marked, with epithelial syncytia and amphophilic to eosinophilic intranuclear inclusion bodies, rhesus macaque (*Macaca mulatta*), primate.

Conference Comment: The contributor provides a concise list of herpesviral diseases of nonhuman primates as well as an excellent review of opportunistic infections in immunocompromised primates. Immunohistochemistry with herpes simplex virus I and II antibodies revealed strong nuclear positivity.

Herpesviruses are composed of an icosahedral shaped nucleocapsid surrounding a double strand of DNA and protein. The nucleocapsid is contained within a lipoprotein envelope which is coated with numerous small glycoprotein peplomers. Herpesvirus virions are fragile and do not survive for long outside the host. Transmission occurs through close or intimate contact, respiratory droplets or transplacentally. Virions attach to host cell receptors via glycoprotein peplomers and the nucleocapsid portion enters the cell through either fusion of the envelope to the cell membrane or by phagocytosis. Once inside the host cell, the DNA-protein complex enters the nucleus, interrupts host cell macromolecular synthesis and initiates the production of its own proteins for construction of more virions. Newly assembled mature virions bud from the nucleus, accumulate within vacuoles in the cytoplasm and are ultimately released by exocytosis or cytolysis. Eosinophilic intranuclear inclusion bodies, visible with the light microscope, are characteristic of herpes virus infections. In general, the alphaherpesviruses typically produce focal lesions in the skin and mucosa of the respiratory or genital tracts. In neonates, alphaherpesvirus infections cause necrosis in a variety of organs and, in pregnant animals, can lead to abortion with necrotic lesions found throughout the fetus. Betaherpesviruses are not typically lytic and cause the formation of greatly enlarged cells (hence the name cytomegalovirus). Whereas gammaherpes viral infections are associated with lymphoproliferative disease and neoplasia. An important characteristic of herpes virus infections is their ability to become latent with intermittent or persistent recrudescence and viral shedding. Alphaherpesviruses are typically latent in nerves; betaherpesviruses in secretory glands, lymphoreticular organs and the kidney; and gammaherpes in lymphoid tissue (15).

A list of common veterinary herpesviral infections is included below. However, herpesviruses have also been found in insects, reptiles, amphibians, mollusks, and exotic mammals, including marine mammals (15).

Alphaherpesviruses

- Bovine:
  - BHV1: Infectious bovine rhinotracheitis
    Infectious pustular vulvovaginitis
    Infectious balanoposthitis
  - BHV2: Bovine mammillitis virus/ Pseudo-lumpy skin disease
  - BHV5: Bovine herpesvirus encephalitis (no inclusion bodies)
- Equine:
  - EHV1: Equine herpesviral abortion, rhinopneumonitis, neurologic disease
  - EHV3: Equine coital exanthema
  - EHV4: Equine rhinopneumonitis, abortion
- Porcine herpesvirus-1: Pseudorabies, Aujeszky's disease
- Canine herpesvirus-1: Canine herpes
- Feline herpesvirus-1: Feline viral rhinotracheitis (FVR)
- Avian:
  - Avian HV1: Infectious laryngotracheitis
  - Avian HV2 (Gallid herpesvirus-2): Marek's disease
  - Anatid HV1: Duck plague
- Nonhuman primate:
  - Herpesvirus simiae (Cercopithecine herpesvirus 1; B virus): Herpes B
  - Herpesvirus tamarinus (Cebid herpesvirus 1; Herpes T): localized disease in squirrel monkeys; generalized disease in marmosets, tamarins, owl monkeys
  - Herpesvirus simplex, type 1: oral lesions in humans, apes, monkeys
  - Herpesvirus simplex, type 2: genital lesions in humans, apes, monkeys
  - Simian varicella: Simian varicella in macaques, African green monkeys, Patas monkeys
  - Herpesvirus papio 2: Oral and genital lesions in baboons

Betaherpesviruses

- Caviid herpesvirus 1: Guinea pig
- Porcine herpesvirus-2: Porcine cytomegalovirus disease/Inclusion body rhinitis

Gammaherpesviruses

- Bovine:
  - Alcelaphine herpesvirus 1: Bovine Malignant Catarrhal Fever
  - BHV4
- Equine:
  - EHV2
  - EHV5

- Nonhuman primates
  - Herpesvirus saimiri (Cebid herpesvirus 2): Squirrel monkey natural host, lymphomas in marmosets, owl monkeys, African green monkeys, howler monkeys and spider monkeys
  - Herpesvirus ateles: Spider monkey natural host, lymphomas in marmosets and owl monkeys
  - Rhesus rhadinovirus: Related to Human herpesvirus-8 (Kaposi sarcoma), possible association with retroperitoneal fibromatosis
  - Rhesus lymphocryptovirus: Lymphoma and epithelial proliferation in immunodeficient hosts
  - Marmoset lymphocryptovirus: Gastrointestinal lymphoma

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CASE II – E1191/05 (AFIP 2987121)

Signalment: 4 years and 10 months old, male, Rottweiler-mix dog (Canis familiaris)

History: The dog was presented to the clinic with a history of recurrent cough for three and a half years associated with fever and diarrhea. Antibiotics, immunostimulating drugs, and a special diet were given. Upon clinical examination, an intense inspiratory sound was present. Radiographically, an interstitial-bronchiolar shadow was found. Endoscopically, hyperemic and slightly edematous mucosal surfaces were observed in the nasopharynx, tonsils, trachea, and bronchi. In deeper airways a mucopurulent exudate was noticed. A foreign body was not detected. Predominantly on the right side, the submucosal tissue appeared granular. Cytological examination of bronchial smears revealed hyperplastic ciliated epithelium, few squamous epithelial cells interpreted as metaplastic cells, numerous goblet cells, few segmented neutrophilic granulocytes and macrophages, as well as mucus. A lobectomy of the right middle lobe was performed, and the tissue was submitted for histopathological examination.

Gross Pathology: The formalin-fixed, submitted lung lobe measured 8 x 5 x 5 cm. The pleura was glossy and smooth. On cut surface multiple cavities ranging from 0.5 to 1.0 cm in diameter were present. The cavities contained viscous, turbid, yellow fluid. Along the dorsal surface of the lobe, a moderate alveolar emphysema was observed.

Laboratory Results: White blood cell counts on four different occasions revealed varying abnormalities: leukocytosis (33,73 x$10^3/\mu$l; ref. range: 5 – 16), mild lymphocytosis (33%; ref. Range 10 – 30), mild granulocytosis (86%; ref. range:

66 –77), and slightly elevated numbers of eosinophilic granulocytes (9%; ref. range 1 4) and monocytes (9%; ref. range: 0 –6).

Microbiological results: A deep airway swab: alpha-hemolyzing and non-hemolyzing streptococci, coagulase-negative staphylococci were isolated.

Histopathologic Description: In the submitted lung tissue single, severely distended bronchi and bronchioles are present. Lumina contain variable amounts of mucopurulent exudate. The ciliated columnar epithelium is hyperplastic and infiltrated with numerous neutrophilic granulocytes and single lymphocytes. In the submucosa, a mild to moderate, diffuse infiltration with lymphocytes and plasma cells is present admixed with few neutrophilic granulocytes and macrophages. In addition, numerous hyperplastic lymphoid follicles and moderate fibrosis are seen. The smooth muscle layer of numerous arteries is moderately hypertrophic. Adjacent alveoli are collapsed and mild alveolar histiocytosis is found.

Contributor's Morphologic Diagnoses: Lung: bronchitis, lympho-plasmacellular and mucopurulent, diffuse, severe, chronic with severe bronchiectasis, epithelial hyperplasia, peribronchiolar follicular hyperplasia, and peribronchiolar atelectasis; moderate hypertrophy of vascular smooth muscles; canine.

Contributor's Comment: Bronchiectasis is an infrequent and debilitating condition of humans and animals, including dogs, cats, cattle, sheep, pigs, mice, and horses[3,11,13]. It is characterized by an irreversible, abnormal dilatation of bronchi. Most often bronchiectasis represents an acquired lesion subsequent to some types of bronchitis. In rare cases it is a congenital malformation. The circumscribed destruction of the airway wall results in focal or diffuse chronic airway dilatation. Accumulation of exudate in the lumen and partial rupture of bronchial walls occur frequently[3].

Grossly, bronchiectasis is characterized by prominent lumps resulting from distension of bronchi and concurrent obstructive atelectasis of the surrounding parenchyma. Bronchiectasis is often mistaken for pulmonary abscesses, because on cut surface dilated bronchi are filled with purulent exudate[14].

Morphologically, two types of bronchiectasis are distinguished, saccular or cylindrical. Bronchiectasis is regarded as saccular when only a small localized portion of the bronchial wall is destroyed with development of thin-walled circumscribed outpouchings. This type of bronchiectasis is less common. It can result from focal necrotizing bronchitis and is described in sheep, cattle and occasionally in horses with chronic obstructive pulmonary disease. Cylindrical bronchiectasis is characterized by destruction of a large bronchial segment or along its entire length. It is localized frequently in the cranio-ventral parts of the lung,

which had been affected by chronic suppurative bronchitis[3]. Bronchiectasis rarely occurs as a congenital malformation.

In cattle, sheep, and pigs, bronchiectasis is acquired after severe broncho-pneumonia or is associated with severe parasitic bronchitis in sheep, goats and pigs[3]. Pathogenically, suppurative bronchitis causes damage to and weakening of the bronchial wall by neutrophilic lysosomal enzymes and oxygen radicals. The accumulation of inflammatory exudate results in obstruction of lower airways followed by alveolar atelectasis. Therefore, inspiration causes traction on the walls of the airways resulting in additional expansion of the lumina. The airflow is less rapid and muco-ciliary clearance is ineffective due to ciliary damage contributing to the accumulation of exudate in the lumen.

In dogs, bronchiectasis usually results from chronic inflammation associated with chronic bronchitis, foreign body or neoplasm[3]. In dogs, atelectasis is a less likely a sequelae of airway obstruction, because there is a very effective collateral ventilation. This may explain that bronchitis is a less frequent cause of bronchiectasis in this species or is limited to only one or two lobes, more often the middle lobes[3]. Other pulmonary lesions including allergic lung disease may also contribute to bronchiectasis in dogs[9,17]. An increased risk of bronchiectasis has been reported in American Cocker Spaniels, West Highland White Terriers, Miniature Poodles, Siberian Huskies, English Springer Spaniels, and dogs over 10 years of age[9].

In cats bronchiectasis was more frequently found in older male individuals and mostly restricted to a single caudal lung lobe. Concurrent respiratory diseases in cats included chronic bronchitis and bronchiolitis, neoplasms, bronchopneumonia, endogenous lipid pneumonia, and emphysema[17]. Heaves, a condition in horses associated with chronic airway inflammation and mucous accumulation[13], may be accompanied by bronchiectasis.

Bronchiectasis with congenital conditions has been described in several breeds of dogs with primary ciliary dyskinesia (immotile cilia syndrome)[1,2,4,5,6,7,8,10,12,15,18,19]. Littermates with this condition have been recognized in English pointers[15], English Springer Spaniels[6], and Old English Sheepdogs[18]. Breeding studies suggest that it is an autosomal recessive trait. In humans, approximately 50% of the patients with primary ciliary dyskinesia have Kartagener's syndrome (situs inversus or partial lateralizing abnormality, sinusitis, and bronchiectasis). Similarly, about 50% of dogs with primary ciliary dyskinesia are affected by situs inversus[1,3]. These dogs usually develop bronchiectasis as a part of abnormalities associated with a basic ciliary defect. A variety of other clinical syndromes are seen with ciliary dyskinesia such as sinusitis, rhinitis, and recurrent bronchopneumonia. Due to the impaired mucociliary clearance subsequent bacterial infections are seen[1]. A common finding in dogs with primary ciliary dyskinesia is communicating hydrocephalus[2,5,6,7,18]. Males are infertile because the tails of spermatozoa are modified cilia, and therefore sperms are often dysmotile[1,5,15,18].

The basic ciliary defect is usually associated with one of several ultrastructural abnormalities of cilia throughout the body, including absence of one or both of the inner and outer dynein arms, microtubular transposition, random microtubular orientation, and partial microtubular deficiency[15].

Definitive diagnosis of primary ciliary dyskinesia usually requires ultrastructural analysis of respiratory cilia, but the fact that several body systems are affected often permits a probable diagnosis to be established using other diagnostic techniques. Interestingly, it has also been noted that some dogs with primary ciliary dyskinesia have no ultrastructural ciliary lesions[5,7].

AFIP Diagnoses: Lung: Bronchiolitis, lymphoplasmacytic, diffuse, chronic, severe, with bronchiolectasis, bronchiolar epithelial hyperplasia, vascular smooth muscle hypertrophy and interstitial fibrosis, Rottweiler-mix, canine.

Conference Comment: The contributor provides an excellent review of bronchiectasis. In this case, the damaged airways present in the sections examined are bronchioles prompting the use of the term bronchiolectasis.

Conference attendees discussed the pathogenesis of bronchiectasis and the complex role of ciliary clearance in the development of respiratory disease. In short, bronchiectasis occurs as a result of the accumulation of exudate in the lumen and partial rupture of bronchial walls. Proteolytic enzymes released from phagocytic cells during chronic inflammation degrade and weaken smooth muscle and cartilage that normally help maintain bronchiolar diameter. The result is destruction of the bronchial and, in this case, bronchiolar walls (20).

Dogs with primary ciliary dyskinesia (PCD) have congenitally impaired mucociliary clearance. This results in the congestion of upper and lower airways with mucus rendering them more susceptible to repeated bacterial infections. Initial signs include a mucoid to mucopurulent nasal discharge, sneezing and coughing (7).

Findings in dogs with PCD include chronic rhinitis characterized histologically by infiltration of the nasal mucosa with plasma cells and neutrophils. Additionally, submucosal mucous glands can become hyperplastic. Other lesions of the upper airways include hypoplastic nasal turbinates, frontal sinusitis, atresia of the frontal sinuses and rhinoliths. Lower airway lesions range from mild bronchitis and bronchiolitis to severe bronchopneumonia, bronchiectasis and ventral lung lobe consolidation (7).

The rhythmic, coordinated beating of cilia is a complex process whose mechanics go beyond the scope of this text. As students of pathology; however, it is important to familiarize ourselves with the ultrastructure of cilia in order to recognize the more common causes of ciliary dyskinesia. In short, cilia are covered

by a ciliary membrane which surrounds the axenome or core. The core is composed of nine outer microtubule doublets surrounding two single, centrally located microtubules (remember the 9, 2 + 2 memory aid i.e. nine outer doublets plus two inner microtubules). Each outer doublet is made up of an A-microtubule and a B-microtubule. The A-microtubule has paired inner and outer dynein arms which project toward the B-microtubule of the adjacent doublet. Additional structural proteins include nexin links which bridge the gap between adjacent doublets and radial spoke linkages which project inward from the A-microtubule toward the center and terminate in a bulbous spoke head (7). In humans with PCD, the most common abnormalities are absence of inner and/or outer dynein arms, transposition of microtubules and defective radial spokes (18). The most commonly reported lesions in dogs include dynein arm deficiency, abnormal microtubular patterns, random orientation, and electron-dense inclusions in the basal body. The basal body, located at the base of the cilia, is derived from a centriole and has nine peripheral microtubule triplets that give rise to the doublets of the axenome. The basal body is anchored to the cell by a striated rootlet and has a lateral projection called the basal foot process (7).

In this case, a picture is worth a thousand words and students are encouraged to study pictures of cilia ultrastructure and become familiar with the complex anatomy. References 7 and 18, as well as several of the others, have excellent diagrams and electron micrographs to help in the understanding of ciliary anatomy, function and dysfunction.

Although ultrastructural analysis can be helpful in diagnosing PCD when lesions are identified in a large percentage of cilia from different locations; in some dogs with PCD no ultrastructural lesions are observed (7).

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CASE III – #8568 (AFIP 2985462)

Signalment: 16 month, female, Multimammate rat, *Mastomys natalensis*

History: A 16 month old female multimammate rat, *Mastomys natalensis*, that was part of a breeding pair, was initially noted to have a small subcutaneous lesion in the right axillary region that slowly progressed to a 2.5 centimeter in diameter firm, multilobular mass. Due to the rat's recent poor reproductive performance, surgical intervention was not considered. The rat was euthanized and submitted for pathological examination.

Gross Pathology: Within the subcutaneous tissue there is a firm, circumscribed, multilobular, tan mass approximately 2.5 cm in maximum diameter.

Histopathologic Description: Within and expanding the subcutis of the ventral skin and extending to the cut border is a 2x1 cm, unencapsulated, but well delineated, lobulated, infiltrative, and densely cellular neoplasm. The tumor is composed of epithelial and spindle-shaped cells arranged in a solid and closely-packed acinar pattern, as well as a stellate to spindloid pattern, respectively. The neoplastic epithelial cells are arranged predominantly as a solid mass of disorganized lobules occasionally forming small acini supported by a highly vascular fibrous stroma. Irregular structures suggestive of ducts of varying size are interspersed throughout the neoplasm. There are variably sized cystic structures lined by pleomorphic acinar cuboidal epithelium filled with eosinophilic proteinaceous secretions and some necrotic cellular debris. Morphologically the neoplastic epithelial cells range from cuboidal to columnar with marked pleomorphism, eccentric heterochromatic nuclei, occasional prominent nucleoli, a high nucleus to cytoplasm ratio, and a moderate mitotic rate of zero to four mitotic figures per 40-power field.

There are scattered interspersed regions of neoplastic spindle-shaped cells projecting into the solid mass where the cells form streams or bundles. The neoplastic spindle cells exhibit indistinct cell borders, oval to spindle-shaped nuclei with blunted ends, one or more distinct nucleoli, clear, crisp cytoplasmic vacuoles, and occasional multinucleate cells. Mitotic figures vary from 1-6 per 40-power field. Additional features of the neoplasm include multiple foci of necrosis with scattered neutrophils surrounded by fibroplasia.

Immunohistochemical staining of the epithelial neoplastic cells was negative for alpha smooth muscle actin (SMA), vimentin, and cytokeratins 5/6 (C5/6).

Cytokeratin CAM 5.2 antibodies resulted in a strong positive staining of tumor cells except for an area of faint staining in one nodular region. Tumor cells were variable in their staining for desmin with the majority of cells exhibiting negativity. The large bands of fibroblasts-like cells stain differently from the main tumor and are positive for SMA, negative for C5/6, and variably positive for CAM 5.2, vimentin and desmin.

Contributor's Morphologic Diagnosis: Mammary gland carcinoma with epithelial to mesenchymal transition

Contributor's Comment: Multimammate rats (*Mastomys natalensis*), sometimes referred to as multimammate mice, are widely distributed rodents of the family Muridae native to African savannas and sub-Saharan regions.[1] Over their 3 year life span these rats breed as monogamous pairs with 6-12 pups per litter. As described in their common name, they typically possess 12, and as many as 18 pairs, of mammary glands. Other unique physiological characteristics include submaxillary salivary glands rich in nerve growth factor[2], prostate glands in both males and females[3], and the absence of a gall bladder. In addition to being susceptible to *Yersinia pestis*, *Mastomys* are natural hosts for leishmaniasis, Lassa virus, and leptospirosis and consequently are commonly used in research involving these agents. Multimammate rats spontaneously develop gastric carcinoids and serve as an animal model for human Zollinger-Ellison syndrome.[4]

Spontaneous mammary gland tumors are common neoplasms in rodents. Variations in cell types of origin, frequency of occurrence and malignancy exist among species and individual strains. Although neoplasia is rare in guinea pigs (*Cavia porcellus*), mammary tumors are represented most frequently by fibroadenomas and ductal adenocarcinomas.[5] In a study of 634 female Djungarian hamsters, (*Phodopus campbelli)* greater than 11% were found to have mammary adenocarcinomas.[6]

Mammary tumors are the most frequently occurring neoplasms in most stocks and strains of rats. In Sprague-Dawley stocks the incidence can approach 50% whereas Fisher 344 rats show an incidence of 15%.[7] Benign fibroadenomas in aged females are the predominant (80-90%) tumor type seen in female Sprague-Dawley rats. In addition to sex, age and genetics, dietary and endocrine factors have been shown to play a role in their occurrence.[8] A reduction of food intake by 20% has been shown to decrease the incidence of mammary tumors in Sprague-Dawley rats by 80%.[9] Ovariectomy markedly reduces tumor incidence and, in one study, prolactin levels were 25 times higher in rats with mammary tumors than in 6-month-old female controls.[8,10] Adenocarcinomas of the mammary gland occur infrequently in the rat.[8]

A variety of factors have been shown to cause mammary tumors in mice; viruses, chemical carcinogens, radiation, genetics, immune status, diet, and hormones.[11] Such tumors are relatively rare in BALB/c, C57BL, and AKR mice, while C3H, DBA/2, and A mice have a high prevalence for spontaneous mammary tumor development.[12] Unlike in humans where metastasis typically involves bone and draining lymph nodes, metastatic lesions secondary to murine mammary tumors are primarily pulmonary in nature.[13,14]

Classification of spontaneous mammary tumors in mice has evolved through several schemes over the last 45 years. In an initial attempt malignant murine mammary tumors were classified as carcinoma, carcinoma with squamous differentiation, and carcinosarcoma. Carcinomas were then further subdivided into adenocarcinoma types (A, B, C, Y, L, and P) with types A and B most common. Type A is represented by adenomas along with tubular and alveolar carcinomas. Type B tumors exhibit regions with varying degrees of differentiation within the same mass. Local invasion and pulmonary metastasis characterize both types.[15] A later system classified tumors according to tissue type (alveolar, ductal, and myoepithelial). Both systems have proven to be insufficient in defining the numbers of mammary tumors recently seen with the emergence of genetically engineered mice. As a result a panel of veterinary and medical pathologists convened in 2000 to produce a scheme based on descriptors and modifiers.[16]

Nomenclature for Histopathological Description of Mammary Gland Pathology in Genetically Engineered Mice

	Descriptors
Descriptors	**Definition**
Glandular	Tumor is composed of glands
Acinar	Tumor is composed of small glandular clusters with small lumens. While this is a subclass of glandular, it is very characteristic of MMTV-induced tumors
Cribriform	Tumor is composed of sheets or nests of cells forming lumens with round, punched out spaces
Papillary	Tumor has finger-like projections composed of epithelium covering a central fibrovascular core
Solid	Tumor is composed of solid sheets of epithelial cells with little or no glandular differentiation
Squamous	Tumors composed solely of squamous cells with or without keratinization, absence of glandular pattern
Fibroadenoma	Tumor is composed of a proliferation of both myxoid fibrous stroma and glands
Adenomyoepithelioma	Tumor is composed of myoepithelium and glands
Adenosquamous	Tumor has both glandular and squamous differentiation

Not Otherwise Specified (NOS)	Tumor does not have any of the other common descriptor

Modifiers

Modifiers	**Definition**
***Biological potential*:**	
Carcinoma	Neoplasm originating from epithelium with proven malignant biological behavior
Adenocarcinoma	Neoplasm originating from glandular epithelium with proven malignant biological behavior
Adenoma	Neoplasm originating from glandular epithelium without proven malignant biological behavior
Mammary Intraepithelial neoplasia (MIN)	Spectrum of intraluminal epithelial proliferations with cytologic atypia including in situ carcinomas
Hyperplasia	Any increase in cell number without cytologic atypia
Tumor	Any space occupying mass with unknown biological potential
***Property*:**	
Atypia	Cells with abnormal nuclear morphology
Necrosis	Cell death generally not applied to programmed cell death (apoptosis)
Fibrosis	Increased or abnormal deposition of connective tissue
Secretory	Tissues or glands producing and exporting lipid or protein
Metaplasia	A change from one adult cell type to another adult cell type
***Topographic*:**	
Diffuse	All of the mammary gland is involved in the process
Focal	One area of the mammary gland is involved in the process
Multifocal	Multiple foci are in the mammary gland
***Inducer (Etiology)*:**	
Gene-induced	Tumors that have morphological or cytological patterns characteristic of specific transgenes or specific mutations. (myc-type, ras-type, erb B2- type)
MMTV-induced	Tumors known to be induced by the mouse mammary tumor virus (MMTV)
Chemically-induced	Tumors known to be induced by a chemical carcinogen
Hormone-induced	Tumors known to be induced by exogenous hormones
***Biological/Experimental context*:**	
Biological	Parity, pregnancy, lactation, involution, hormones
Experimental	Promotor, exogenous hormones or chemicals

Source: Cardiff RD et al. (2000)

Recently in 2004, mouse mammary tumors were characterized by morphology and immunohistochemistry for expression of terminal differentiation and categorized as

'simple' carcinomas, 'complex' carcinomas and carcinomas with epithelial to mesenchymal transition (EMT) markers.[17]

Mammary neoplasia, common in mice and rats, has been shown to be rare or absent in surveys of spontaneous disease in *Mastomys natalensis*.[18,19] In this instance a solitary mammary tumor was present with no evidence of pulmonary or lymph node metastasis. Immunohistochemical staining for different components of the cytoskeleton such as intermediate filaments (e.g. cytokeratins and vimentin) and actin microfilaments can be used to determine mesenchymal from epithelial cell types of origin.

Keratins consist of a group of 19 polypeptides of molecular weights between 40-67 kilodaltons and serve as reliable markers for epithelial cell differentiation. Subsets of keratins are expressed variably as cells differentiate. Carcinomas generally express cytokeratin patterns reflective of the cell type of origin. Non-epithelial tumors are most frequently cytokeratin negative although few mesenchymal neoplasms may show faint or focal positivity to cytokeratins 8/18/19. In humans, breast myoepithelial cells express cytokeratins 5/14. In luminal and secretory mammary epithelia expression of cytokeratins 8/18 predominate.[20] Therefore antibody binding to selected cytokeratins can aid in differentiating myoepithelial from ductal or lobular mammary carcinomas.

In this case, negative staining for vimentin rules out the likelihood of a mesenchymal precursor. Lack of staining for both alpha smooth muscle actin and cytokeratins 5/6 in the presence of a strong positive antibody response to cytokeratin CAM 5.2 suggests a secretory rather than a myoepithelial immunophenotype. Desmin is a marker for intermediate filaments of skeletal, cardiac, and smooth muscle. Although shown to be expressed in a percentage of human non-myogenic tumors such as malignant fibrous histiocytoma, fibromatosis, and lung carcinomas a variable positive staining for desmin in this case suggests the presence of compartments of less differentiated cells.[17,21] Cytokeratin CAM 5.2 staining recognizes cytokeratins 8/18 which are associated with ductal and lobular cell types. A strong positive staining along with the presence of ducts within the tumor suggests a ductal cell type of origin. The subpopulation of neoplastic spindle-shaped cells with strong positive staining for SMA, negative staining for C5/6, and variably positive staining for CAM 5.2, vimentin and desmin suggest mesenchymal differentiation. The presence of two populations of cells, one epithelial and one mesenchymal, suggest that this tumor may be of mammary stem cell origin.

AFIP Diagnosis: Mammary gland (per contributor): Adenocarcinoma, Multimammate rat (*Mastomys natalensis*), rodent.

Conference Comment: The contributor provides an excellent and in-depth review of the naming scheme and immunohistochemical findings associated with rodent mammary neoplasms.

Epithelial to mesenchymal transition (EMT) and its role in tumor metastasis is a concept which has recently sparked some debate. The process of epithelial to mesenchymal transition was first described in 1982 with the discovery that epithelial cells in culture may acquire mesenchymal features. EMT has been described as a feature of embryonic development as well as the development of malignant and chronic fibrotic disorders and in cancer progression, specifically with tumor invasiveness and intravasations and extravasations of metastatic cells. More specifically, EMT involves a complex, extreme manifestation of epithelial plasticity where polarized epithelial cells embedded in organized stratified or single cell layers convert into single fibroblastoid cells capable of locomotion. This occurs in a series of events which include the release of cells from epithelial polarity, remodeling of epithelial cell-cell and cell-matrix adhesion contacts and of their actin cytoskeleton (22).

The crux of the debate lies in whether or not EMT is truly a reflection of a change in cell lineage or whether it merely reflects a change in the phenotype of cells actively involved in metastasis. It has been argued that the metastatic spread of cancer from the primary site to form secondary tumors in distant organs constitutes the most stringent test of the EMT hypothesis and that for this to occur the neoplastic cells would have to undergo reverse EMT or mesenchymal-to-epithelial transition once they reached the target organ and formed a secondary tumor (23).

It is important to be clear, however, that this skepticism about EMT in malignancy in no way challenges the role of epithelial mesenchymal interactions in development, healing and remodeling, carcinogenesis, and metastasis, for which there is substantial evidence. The skepticism is directed towards transmutation of one lineage into another, not between biologically important interactions between them (23).

While it is important to familiarize ourselves with the current concept of epithelial to mesenchymal transition and the debate which currently surrounds the idea, it is beyond the scope of this text to completely decipher the intricacies of this complex process. Certainly it qualifies as a topic better left to oncologists rather than your garden variety anatomic pathology resident.

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CASE IV – MK01-2477 (AFIP 2992271)

Signalment: Adult, male, rhesus macaque, *Macaca mulatta*

History: This animal was challenged with simian immunodeficiency virus, SIV, seven days prior to necropsy, and was started on 9-R-(2-phosphonomethoxypropyl)adenine (PMPA, or tenofovir) antiviral treatment a day after he was challenged with SIV. He was then found lethargic the day before necropsy, and found dead in his cage the following morning. A total of six days elapsed between initiation of the PMPA therapy and death of the animal.

Gross Pathology: At necropsy, this animal appeared well muscled with a moderate amount of body fat and in good hydration. Upon reflecting the abdominal skin, hemorrhage and subcutaneous edema were evident. There were also multifocal petechial and ecchymotic hemorrhages present in the skin of the medial legs, thighs, and arms. Both kidneys were mildly pale with soft cortices, and there was a moderate amount of peri-capsular hemorrhage adjacent to the left kidney.

Approximately 100 ml of serosanguineous fluid was present in the thoracic cavity, and approximately 50 ml of clear amber fluid was present in the abdominal cavity. In addition, the liver had an accentuated lobular pattern with an expansile mass present in the right lateral lobe. This mass measured 6 X 5 X 3.5 cm and had a prominent vascular pattern. The lungs were diffusely edematous and exuded serosanguineous fluid from cut surfaces. A moderate amount of white froth was present in the trachea. Within the intestinal tract and colon there were multifocal submucosal and subserosal hemorrhages present. All other tissues appeared grossly to be normal.

Laboratory Results: Samples for bacterial culture were collected from the clear amber fluid present in the abdominal cavity as well as from samples of the right caudal lung lobe and spleen. Bacterial cultures yielded *E. coli* and *Morganella morganii*, with this growth likely representing post-mortem bacterial overgrowth. This animal also had extremely elevated creatinine and BUN levels, which is consistent with the extensive renal lesions.

Histopathologic Description: The sample submitted was from sections taken from the same kidney, most of which contained sections through the cortex, medulla, and renal pelvis. Diffusely there was necrosis of proximal convoluted tubules. Most proximal tubules had lost their tubular epithelium with pink, granular, cellular debris filling the tubular lumens. In other proximal tubules, the epithelium was flattened and attenuated. Some proximal tubules were undergoing regeneration with epithelial hypertrophy and hyperplasia. The glomeruli were uniform in size, and many glomeruli contained eosinophilic, homogenous, condensed material within the lumen of the capillaries. This material was consistent with fibrin or fibrinoid deposits. There were no inflammatory cell infiltrates present.

Contributor's Morphologic Diagnoses:

1. Kidneys: tubular necrosis of the proximal convoluted tubules, bilateral, severe, acute
2. Kidneys: glomerular capillaries – fibrin thrombi, diffuse, severe
3. Liver: nodular hyperplasia

Contributor's Comment: The cause of death in this animal was due to severe acute renal tubular necrosis brought on by acute toxicosis. Given the short time period of infection, the cause of the renal lesions and the eventual death of the animal was not related to the SIV infection, but rather to the treatment with an antiviral agent, PMPA (9-R-[2-phosphonomethoxypropyl]adenine). PMPA, an acyclic nucleotide analogue, is a reverse transcriptase inhibitor that has worked well in clinical trials on both SIV and human immunodeficiency virus (HIV) infections in reducing viremia and improving overall lifespan (7). Since PMPA is only bioavailable parenterally and not orally, tenofovir disoproxil fumarate (TDF),

which was approved by the Food and Drug Administration (FDA) in 2001 for the treatment of HIV, is a prodrug form of PMPA that is bioavailable orally (2) and has been used as part of a highly active antiretroviral therapy (HAART) to treat HIV infected humans (3, 6, 11).

Although other closely related acyclic nucleoside analogue antivirals, such as cidofovir and adefovir, have been associated with nephrotoxicity, tenofovir was not expected to have similar problems (3, 6, 7, 10, 11). Human trials with TDF failed to show evidence of clinically relevant renal toxicity; however, since August of 2003 there have been at least 22 cases of TDF-associated nephrotoxicity with more occurring every year (1).

There are several mechanisms of toxicity from antiviral agents that could explain the nephrotoxicity, including mitochondrial injury and transporter defects; however, tenofovir was not expected to have similar nephrotoxic results as cidofovir and adefovir in humans. This is mainly due to decreased interaction with human organic transporter 1 and minimal mitochondrial DNA toxicity in vitro (5, 10). The actual mechanism of renal injury caused by tenofovir has not been confirmed, although there are several hypotheses. One possibility is that drug interactions are responsible for the nephrotoxicity since tenofovir primarily is excreted by the kidneys via both glomerular filtration and active tubular secretion (3). Several other drugs, including the antiviral drugs acyclovir, cidofovir, ganciclovir, and valacyclovir, also compete for active tubular secretion, which potentially could raise concentrations of tenofovir in the serum (7, 10). In addition, dose-dependency is likely a factor, since nephrotoxicity closely corresponds with both dose and duration of therapy in animal models (7). Either way, once tenofovir reaches high concentrations in the renal tubules, cell function could be disrupted. Besides the drug-related pathways of nephrotoxicity, there are also patient-related factors that must be taken into consideration when considering risk factors. For instance, patients that have previous renal impairment, are dehydrated, are elderly, have electrolyte depletion or are acidotic are more likely to develop nephrotoxicity as the result of antiviral therapy then are young, healthy patients (5).

To date, there have been several clinical manifestations of kidney disease resulting from tenofovir use in humans infected with HIV. Many cases of tenofovir-associated renal toxicity are characterized by Fanconi's syndrome, which is typified by proximal renal tubular dysfunction, hyperaminoaciduria, glucosuria, and phosphaturia (1, 7, 10). In addition, other manifestations have included diabetes insipidus and acute renal failure (5). Often renal tubular epithelial cells and casts are observed in human cases (5). Given the results from subsequent human cases, it is not surprising that this case in 2001 demonstrated acute proximal tubular necrosis after PMPA treatment. In human cases of acute renal failure caused by nephrotoxicity, most toxicity is wholly or partially reversible; however, a large

proportion of the cases will retain some renal damage (4). The case presented, on the other hand, was likely given a dose for which recovery was not possible. This is supported by the rapid onset of renal failure—this animal was lethargic within five days after PMPA treatment was initiated.

In addition to acute proximal tubular necrosis, this animal had fibrin thrombi within the glomerular capillaries indicative of disseminated intravascular coagulation (DIC). Phosphotungstic Acid Hematoxylin (PTAH) staining of the renal sections was positive for fibrin within glomerular capillaries. This animal also had multifocal hemorrhage in the subcutis, skeletal muscle, myocardium, intestine, colon, and adrenal glands, which is consistent with DIC (8, 9). There were no fibrin thrombi evident in other vessels, including the alveolar capillaries. The major mechanisms leading to DIC are widespread injury to endothelial cells and the release of thromboplastic substances into the circulation. Although viremia can lead to DIC, in this case the DIC was not likely secondary to viremia given the short period of time the animal had been infected with SIV. It is more likely that the DIC was secondary to the antiviral treatment with PMPA. Drug therapy can cause blood stasis, leading to excessive platelet activation and a state of hypercoagulation.

AFIP Diagnosis: 1. Kidney, tubules: Necrosis, acute, multifocal, with scattered regeneration, rhesus macaque, primate.
2. Kidney, glomerular capillaries: Thrombi, multiple.

Conference Comment: This is an interesting case of an anti-retroviral drug inducing acute tubular necrosis. The contributor provides an excellent review of anti-retroviral drugs currently in use for the treatment of HIV infection and their nephrotoxic side-effects.

Conference attendees debated the presence of regenerating tubular epithelial cells and the presence of multiple fibrin thrombi occluding glomerular capillaries. Tubular epithelial cell regeneration is characterized by the presence of mitotic figures lining the tubular basement membrane and plump epithelial cells with slightly basophilic cytoplasm. In this case, the proximal convoluted tubules appeared most effected which is often the case with nephrotoxins. The presence of fibrin was confirmed with phosphotungstic acid hematoxylin (PTAH) staining.

Discussion focused on the myriad causes of nephrotoxic tubular necrosis and covered the basic categories of chemical and plant toxins associated with renal disease. Below is a short list of the chemical and plant toxins which most commonly result in tubular necrosis (12):

Pharmaceuticals

- Aminoglycosides-concentrate in lysosomes. When released they result in inhibition of the sodium-potassium-ATPase pump resulting in an intracellular influx of hydrogen and sodium ions and water; also inhibit phospholipase which results in the intracellular accumulation of phospholipids, altered mitochondrial function and inhibition of protein synthesis
- Oxytetracycline-large concentrations inhibit mammalian protein synthesis; tubular obstruction caused by desquamated necrotic tubular epithelium
- Amphotericin B-directly disrupts cell membranes, interferes with cholesterol-lipid interactions and causes potassium ion loss, hydrogen accumulation and cellular swelling and necrosis
- Sulfonamide-induced tubular necrosis-dehydrated animals; mechanical damage and direct toxicity due to crystal formation in tubules
- Monensin-altered ion transport
- Nonsteroidal anti-inflammatory drugs (NSAIDs)-decreased synthesis of renal prostaglandins resulting in arteriolar constriction->reduced renal perfusion->acute tubular degeneration and necrosis
- Cisplatin-directly damages tubules and causes vasoconstriction via renin-angiotensin mechanism->reduced renal blood flow->tubular degeneration and necrosis

Heavy metals

- Inorganic mercury-mercuric ions concentrate in rough endoplasmic reticulum->loss of brush border->mitochondrial swelling->cell death
- Cadmium, inorganic-induces tubular epithelial apoptosis
- Lead-damages mitochondria and epithelial cell membranes
- Thallium-a rodenticide, damages cell membranes
- Arsenic, inorganic-pesticide, damages cell membranes

Plant toxins

- *Amaranthus retroflexus* (Redroot pigweed)-acute tubular necrosis in cattle; perirenal edema in pigs
- *Quercus sp.* (Oak)-toxins are metabolites of tannins; pale swollen kidneys with petechial hemorrhages in cattle, also perirenal edema, abundant clear fluid in body cavities, renal fibrosis.
- *Halogeton, Sarcobatus* (Greasewood), *Rheum, Rumex*-oxalate containing plants, calcium oxalate precipitate in renal tubules->obstruction->necrosis
- *Cestrum diurnum, Solanum sp., Trisetum sp.*-all contain Vitamin D analogs; ingestion results in hypercalcemia->tubular epithelial cell absorption of calcium->mitochondrial calcification and dysfunction->cell death; also vasoconstriction->ischemia

Household toxins

- Ethylene glycol-antifreeze, toxic metabolites cause acute tubular necrosis; calcium oxalate crystals form in tubules and interstitium->obstruction

The focus then shifted to a discussion and review of Virchow's triad of events that predispose to thrombus formation (8). Those events are:

1) Endothelial injury, to include endothelial dysfunction
2) Alterations in normal blood flow such as turbulence and stasis
3) Hypercoagulability, genetic and acquired causes

The conference concluded with a brief overview of the fate of a thrombus once it has formed (8). Once formed, a thrombus can:

1) Undergo **propagation** by accumulating more platelets and fibrin, ultimately obstructing the vessel
2) **Embolize** or dislodge and travel to another location
3) Undergo **dissolution** via fibrinolytic activity
4) Undergo **organization,** by inducing inflammation and fibrosis; and **recanalization**, thereby reestablishing blood flow

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