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CASE I – 01-A-89 (AFIP 2890553)

Signalment: Seventeen year old, female, rhesus macaque, *Macaca mulatta*, nonhuman primate.

History: This animal presented to necropsy as part of a terminal research project. A semi-purified diet had been fed from birth. Chronic, intermittent episodes of vomiting were reported. She was deeply anesthetized and exsanguinated via the distal aorta. A hyperosmolar paraformaldehyde/glutaraldehyde solution was administered via the left ventricle.

Gross Pathology: The distal ten cm of the esophagus were flaccid and dilated to 2.5 to 3 cm. The esophageal mucosa was mildly to moderately thickened throughout its length.

Laboratory Results: None reported.

Contributor's Morphologic Diagnosis: Esophagus: Esophagitis, proliferative, chronic-active, lymphoplasmacytic, eosinophilic, diffuse with gastric and intestinal metaplasia (Barrett's esophagus), glandular abscesses, hyperplasia of the muscularis mucosa and erosions.

Contributor's Comment: The esophageal mucosa is thickened and markedly hyperplastic with prominent downgrowth of epithelial cords. The stratified squamous epithelium of the distal portion of the cords is extensively replaced by glandular epithelium characterized by columnar cells that resemble either intestinal absorptive cells or goblet cells. There is multifocal single cell necrosis of the

glandular lining cells. The glands are frequently dilated and contain neutrophils and/or karyorrhectic debris. In other areas, the surface esophageal mucosa is completely replaced by columnar epithelium that resembles gastric foveolar cells, rarely with parietal cells admixed (not present in all sections). High numbers of plasma cells admixed with fewer lymphocytes, eosinophils, macrophages and neutrophils expand the superficial lamina propria and multifocally traverse the overlying surface epithelium and the glandular epithelium. Multifocally, the mucosa is eroded and there is luminal exudation of neutrophils. The muscularis mucosa is multifocally hyperplastic. The vasculature is diffusely, moderately ectatic.

Barrett's esophagus in humans is generally defined as the presence of metaplastic specialized columnar epithelium occurring in the distal esophagus. Typically, the metaplastic epithelium contains a mix of gastric cardia-type mucosa and intestinal goblet cells.[1,2,3] Incomplete intestinal metaplasia is most often seen in Barrett's esophagus and is characterized by goblet cell metaplasia. Less common is complete intestinal metaplasia or the added presence of intestinal absorptive cells.[2,3] Most cell types found in the gastric and intestinal mucosa (Paneth cells, goblet cells, parietal cells, chief cells, small intestinal absorptive cells, and gastric foveolar cells) may occur in Barrett's esophagus.[3]

Barrett's esophagus in humans is a sequel of chronic gastroesophageal reflux. Although the pathogenesis is unclear, it is thought to be a protective metaplastic response to prolonged mucosal injury. It is of particular clinical concern in humans because it is associated with a significantly increased risk of development of esophageal adenocarcinoma[1,3].

AFIP Diagnoses:
1. Esophagus: Squamous metaplasia of submucosal ducts, focal, marked, with mild lymphoplasmacytic, histiocytic, and eosinophilic esophagitis, and marked smooth muscle hyperplasia, rhesus macaque, nonhuman primate.
2. Esophagus: Intestinal metaplasia, focal, marked.

Conference Comment: This case was reviewed in consultation with the Department of Gastrointestinal Pathology at the Armed Forces Institute of Pathology. There was variation in submitted slides, with two distinct presentations in the slides presented in conference.

The first presentation is that of squamous metaplasia of the esophageal submucosal ducts and pronounced hypertrophy of the muscularis mucosa. This is consistent with a condition in humans called pseudodiverticulosis. The squamous metaplasia leads to blockage of outflow, causing cystic dilation of the glands with

atrophy. If extensive, these can resemble diverticula, hence the name. The Department of Gastrointestinal Pathology noted that, in humans, these are not usually diagnosed with biopsy. These intramural lesions can be seen grossly, and are diagnosed endoscopically or radiographically.[4]

The second presentation is also that of pseudodiverticulosis, but with an abrupt transition to intestinal metaplasia with numerous goblet cells characteristic of Barrett's esophagus. The presence of goblet cells is reported to be the most useful feature for diagnosis of Barrett's esophagus since these are not normally present in the gastric mucosa. The two major components of Barrett's esophagus are metaplasia of the surface and pit epithelium and metaplasia of the mucous glands. The surface and pit epithelium may be lined by a combination of goblet and columnar cells. The mucous glands are usually composed of pure mucous cells, but may also contain parietal cells, Paneth cells, endocrine cells, or pancreatic acinar cells.[4]

Contributor: Oregon National Primate Research Center
http://onprc.ohsu.edu

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CASE II – AR02-373 (AFIP 2888365)

Signalment: 5-month-old male nu/nu mouse (*Mus musculus*).

History: During a 2 week period, four 18-week-old male outbred *nu/nu* mice were found to be tachypneic, lethargic, hunched, and hypothermic, with varying degrees of abdominal distension. The mice were group housed in separate microisolator cages on ventilated racks with corn cob bedding in a barrier facility. These animals were part of a cancer study and had recently received subcutaneous flank

injections of glioblastoma cells. No drug treatment had yet been given. Previous clinical history included several incidences of fighting among cage mates resulting in multifocal superficial puncture wounds of the caudal dorsum. At time of presentation the prognosis was poor and euthanasia was elected.

Gross Pathology: An adult male nude mouse in slightly thin body condition was examined. Weight at necropsy was 22.2 grams. No postmortem changes were evident. Diffusely the subcutis was expanded by abundant clear gelatinous material (edema) (Fig. 1), and the thoracic and abdominal cavities each contained ~0.5ml of clear, red-tinged fluid. The pancreatic lobules were markedly separated by clear fluid. Both kidneys were pale brown with a finely granular surface. Ingesta was present in the stomach. No masses were evident on either flank.
Gross Morphologic Diagnoses:

Nephropathy, Bilateral, Chronic, Moderate
Anasarca

Histologic Findings:
Kidney: The glomerular capillary tufts are segmentally expanded by abundant homogenous, pale eosinophilic infiltrate which is uniformly populated by deeply basophilic, pleomorphic, angular, and karyorrhectic nuclei. Bowman's capsule contains cells with similar, but distinctly vesicular, nuclei and pale amphophilic cytoplasm. Each corpuscle is circumferentially delineated by 2-5 layers of fibrous connective tissue. The cortical and medullary interstitium is diffusely hypercellular owing to large numbers of lymphocytes and plasma cells with occasional mast cells. Congo red stain for amyloid is negative.

Ultrastructural Findings: (Fig. 2)
Transmission electron microscopy revealed segmental expansion of the glomerular basement membrane and mesangium by large deposits of medium electron dense material containing linear arrays of denser fibrils. These fibrils were arranged in packets of long, wavy, roughly parallel rows suggestive of membranous lamellae, but lacked consistent periodicity. Occasional podocyte foot processes were blunted and fused.

Laboratory Results: None reported.

Contributor's Morphologic Diagnosis: Membranous Glomerulonephropathy, Diffuse, Chronic, Severe with Periglomerular Fibrosis and Moderate Subacute Lymphoplasmacytic Interstitial Nephritis, Kidney.

Contributor's Comment: This condition has previously been documented as a sporadic finding in athymic nude mice and associated with circulating antinuclear antibody.[1] In general, two types of immune-mediated glomerulonephritis are

recognized, and classification is based on the immunologically perceived antigen. In immune complex glomerulonephritis, antigen-antibody complexes are passively deposited in the glomeruli. Conversely, autoimmune glomerulonephritis involves active binding of antibody to renal antigens, such as basement membrane or mesangial cells.[2] The findings of Pelletier et al suggest that the lesion in this case is secondary to deposition of circulating autoantigen-autoantibody complexes. The role of experimental injection of tumor cells in this case is unclear, but tumor-associated inflammation may have contributed to the pathogenesis.

AFIP Diagnosis: Kidney: Glomerulonephritis, membranous, global, diffuse, severe, with tubular ectasia and protein casts, nu/nu mouse, rodent.

Transmission electron micrograph: Glomerulonephritis, membranous, with intramembranous irregularly arranged, electron dense fibrils.

Conference Comment: Conference attendees discussed the ultrastructural appearance of different types of glomerular deposits. Amyloid deposits are characterized by irregular, non-branching fibrils that are 10-15nm in diameter.[4] Collagen consists of well-organized fibrils with periodic cross-banding. The width, periodicity, and arrangement of the fibrils depend on the type of collagen.[3] Immune complexes consist of electron dense granules within or on either side of the glomerular basement membrane.[4] While the appearance of the immune complexes in this case consisting of well-structured, parallel fibers is unusual, it is interesting to note that this pattern of glomerular deposits has also been reported in humans with systemic lupus erythematosus.[2,3]

Contributor: Wake Forest University School of Medicine
http://www.wfubmc.edu

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CASE III – 01-2452 (AFIP 2888758)

Signalment: Porcine, mix breed, 36 day old, intact male.

History: Caesarian-derived, gnotobiotically maintained piglets were experimentally inoculated on day 1 of life with $4.3x10^6$ units of infectious porcine circovirus-2 (PCV-2) by oral-nasal route[2]. Piglets were given 50 mg/kg/days 1-5 and 25 mg/kg/days 6 through termination of cyclosporin (Neoral ®) *per os*. This piglet was normal until day 35 of life at which time he became lethargic and icteric. He was afebrile. The pig was euthanatized on day 36 of life.

Gross Pathology: In addition to icterus, the liver was small and ascites was present.

Laboratory Results: Compared with virus infected non-cyclosporin-treated controls, virus infected piglets treated with cyclosporin had significant lymphopenia and hypoproteinemia which was characterized by lowered albumin alpha and beta-globulin and absence of gamma-globulin.

Contributor's Morphologic Diagnosis: Liver: Marked widespread to diffuse subacute hepatocellular necrosis with hepatocellular loss.

Contributor's Comment: This experiment was conducted in order to determine the effects of immunosuppression on the pathogenesis of PCV-2 infection in piglets, and the role of the immune response in lesion development[1]. PCV-2 is the suspected cause of Porcine Multisystemic Wasting Syndrome. Infection of cyclosporin-treated gnotobiotic piglets resulted in diffuse hepatocellular necrosis with minimal inflammatory response. There was no hepatic necrosis in piglets given virus alone. Significant amounts of virus were present in livers of PCV-2 infected, cyclosporin-treated piglets compared with PCV-2 infected, untreated piglets. Hepatocytes and Kupffer cells contained both intranuclear and intracytoplasmic viral antigen. Intracytoplasmic inclusion bodies in Kupffer cells in these slides are very difficult to distinguish from phagocytized debris. In non-immunosuppressed piglets, PCV-2 virus accumulates in the cytoplasm of macrophages, mononuclear cells, and histiocytes, disseminates via monocytes and serum/plasma viremia, causes minimal or no overt viral cytopathic effects, and induces angiocentric granulomatous inflammatory cell infiltrates that can lead to organ failure[2]. Viral antigen is rarely detected in hepatocytes in viral infected non-immunosuppressed piglets[2]. It is suspected that immunosuppression of these piglets by cyclosporin allowed for an increased, sustained viremia with infection of Kupffer cells, and secondarily, of hepatocytes. The lack of significant inflammatory response to the infection of the liver suggests that virus infection of hepatocytes

overwhelmed cell function, leading to cell death[1].

AFIP Diagnosis: Liver: Hepatocellular degeneration, necrosis, and loss, diffuse, with stromal collapse and cholestasis, mixed breed, porcine.

Conference Comment: The differential diagnosis for hepatic necrosis discussed by conference attendees included hepatosis dietetica, aflatoxicosis, cocklebur intoxication, gossypol intoxication, pyrrolizidine alkaloid toxicity, and porcine circovirus-2 (PCV-2) infection. Although there was not striking biliary hyperplasia, some attendees preferred a toxic etiology as they considered the large vacuolated cells to represent megalocytosis. After discussion, it was concluded that these large vacuolated cells probably represent a degenerative change.

Postweaning multisystemic wasting syndrome (PMWS) causes generalized lymphadenopathy, hepatitis, nephritis, and pneumonia in piglets. It is reported that immune activation is a key component in the pathogenesis of PCV-2-associated PMWS in pigs. Coinfection with other pathogens, such as porcine parvovirus and porcine reproductive and respiratory syndrome virus (arterivirus), have been found to be required to cause clinical PCV-2 disease in gnotobiotic piglets.[1,3,4] Other exacerbating factors include overcrowding, co-mingling of age groups, and other stressors.[3]

Typical gross findings include marked systemic lymphadenopathy, hepatitis with icterus and edema, and firm, mottled lungs that fail to collapse. Histologically, there is lymphoid depletion with infiltration by histiocytes and multinucleated giant cells with intensely basophilic botryoid intranuclear inclusion bodies. There is also granulomatous hepatitis, interstitial pneumonia, and nephritis.[3]

Contributor: Department of Veterinary Biosciences, The Ohio State University http://www.vet.ohio-state.edu/level2/depart/depart.html

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wasting syndrome in gnotobiotic swine by coinfection with porcine circovirus 2 and porcine parvovirus. Vet Pathol **37**:254-263, 2000

CASE IV – 30927 (AFIP 2910190)

Signalment: Adult, male African green monkey (*Chlorocebus aethiops*), nonhuman primate.

History: This 6.9 kg African green monkey was exposed to a lethal dose of ricin toxin by inhalation*****. The animal died approximately 72 hours postexposure. The body presented for necropsy is that of an adult, male African green monkey (*Chlorocebus aethiops*). The carcass is in good body condition with adequate subcutaneous and cavitary fat. The pelage is within normal limits.

***** Research was conducted in compliance with the Animal Welfare Act and other Federal statutes and regulations relating to animals and experiments involving animals and adheres to principles stated in the *Guide for the Care and Use of Laboratory Animals*, National Research Council, 1996. The facility where this research was conducted is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International.

Gross Pathology: Gross examination reveals a missing left index finger. There is approximately 30-60 ml of serosanguineous pleural effusion in the thoracic cavity, and the lungs are moderately congested, hemorrhagic, and edematous. (Figs. 1, 2) The stomach contains a small amount of ingesta. There is scant material in the small intestine, with normal-appearing fecal material within the colon and rectum. The gall bladder is full of bile and the urinary bladder contains urine.

Gross diagnoses:
1. Lung: Hemorrhage, congestion, and edema, diffuse, moderate, with serosanguineous pleural effusion.
2. Left hand: Missing digit #2 (index finger).

Laboratory Results: None reported.

Contributor's Morphologic Diagnoses:
Microscopic diagnoses:
1. Lung: Pneumonia, fibrinohemorrhagic, acute to subacute, diffuse, moderate, with edema, and multifocal septal necrosis, vasculitis, and fibrinous pleuritis, African green monkey (*Chlorocebus aethiops*), nonhuman primate.

The following sections are not provided, but diagnoses are included for completeness:

2. Mediastinum: Mediastinitis, neutrophilic and histiocytic, multifocal, moderate, with hemorrhage, fibrin and edema.
3. Lymph node, tracheobronchial: Lymphadenitis, neutrophilic, acute, multifocal, mild, with draining hemorrhage and edema.
4. Heart, left ventricle: Myocarditis, acute to subacute, multifocal, mild, with myocyte necrosis.

Contributor's Comment**: Ricin is a highly toxic protein derived from the bean of the castor oil plant, *Ricinus communis*, composed of two polypeptide chains joined by a disulfide bond. The active A chain is the toxic moiety, and the B chain is the binding lectin moiety. Rapid uptake of the toxin occurs after B chain binding to glycoprotein cell-surface receptors.[3] Transport of the A chain moiety to cellular ribosomes results in catalytic inhibition of protein synthesis by inactivation of the 28s ribosomal subunit. Toxicity is dependent on dose and route of exposure.[1]

Ricin has been used as a weapon of terrorism and assassination and is considered a potential biological warfare threat agent to military operations.[2] Aerosol ricin exposure conducted in rats and rhesus monkeys results in signs of respiratory embarrassment within 8-24 hours depending on exposure dose. Grossly, lesions are confined to the respiratory tract and consist of serous to serosanguineous fluid within the thoracic cavity. Lungs are edematous and heavy, do not collapse, and are mottled red and purple. Fibrin strands are occasionally attached to pleural surfaces. Histologically, there is multifocal to coalescing areas of intra-alveolar fibrin, edema, and hemorrhage, acute alveolitis, and necrosis of lower respiratory tract epithelium.[3]

Although it is suspected that ricin-induced pulmonary edema is due to increased pulmonary capillary endothelium permeability or 'vascular leak syndrome', the specific mechanism by which inhaled ricin crosses the respiratory epithelium to injure the vascular endothelium has not yet been determined.[3]

In this study, the pulmonary lesions and the related hemorrhage and inflammation in draining lymph nodes and mediastinum are consistent with reported inhalation ricin toxin exposure. The lesions in the draining lymphatics and surrounding tissues may have been induced by transported intact ricin molecules detached from cells, or ricin-stimulated effector cells or inflammatory cytokines.[3] The mild inflammation also seen in the heart is possibly related to cardiopulmonary insufficiency induced by the toxin.

**Opinions, interpretations, conclusions, and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

AFIP Diagnosis: Lung: Pneumonia, interstitial, necrotizing, acute, diffuse, severe, with hemorrhage, fibrin, and edema, African green monkey (*Chlorocebus aethiops*), nonhuman primate.

Conference Comment: The contributor gives a concise overview of ricin toxicosis. While all parts of the castor bean plant are toxic, the beans contain the most ricin and must be crushed or broken to release the toxic component. Ricin is 100 times more toxic parenterally than orally. Ingestion causes vomiting, abdominal pain, diarrhea, and gastrointestinal hemorrhage. After parenteral administration, ricin causes hemorrhage and necrosis in the heart, stomach, lungs, liver, kidneys, and pancreas.[5]

Two other toxic causes of pulmonary edema were discussed during the conference. Oxygen toxicity causes damage to type I pneumocytes and capillary endothelium, with hyaline membrane formation from cellular debris and proteinaceous exudate.[4] Paraquat poisoning is characterized acutely by hemorrhage, hyaline membrane formation, and loss of pneumocytes. With chronic insult, diffuse interstitial and intraalveolar fibrosis and type II pneumocyte hyperplasia develop.[4]

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