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Department of Veterinary Pathology
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CONFERENCE 5
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Conference Moderator: Dr. Tim O'Neill
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Maryland Department of Agriculture
Frederick, MD 21702

CASE I – 01-1890-6 (AFIP 2788785)

Signalment: Adult, male, cynomolgus macaque (*Macaca fascicularis*)

History: This macaque developed a fever of unknown origin approximately 28 days post surgery for transplantation of a transgenic piglet heart into its abdomen. The monkey became depressed, anorexic, and developed diarrhea at day 31. The immunosuppressive drug regime utilized previously in xenograft transplant monkeys consisted of a combination of cyclosporine, methylprednisolone, and D4ERL, but in this monkey, antithymocyte globulin (ATG) was also added. Therapies initiated at the onset of clinical signs included Timentin, Gentocin, Fluconazole, metronidazole, and one dose of gancyclovir. Because of the animal's deteriorating condition, the investigators agreed to euthanasia.

Gross Pathology: Gross lesions consisted of rare petechial hemorrhages in the lung and kidney, and mild swelling and pallor of the kidneys.

Laboratory Results: Pre-surgical screening for Herpes B, SIV, SRV, STLV and TB were all negative. At the time of euthanasia (day 36) serum collected was positive for simian Cytomegalovirus. PCR on plasma and lung (generously run by Dr. Peter Barry, UC Davis) using rhesus CMV (RhCMV) primers, were negative. Immunohistochemistry (also performed by Dr. Peter Barry) on formalin fixed, paraffin embedded lung was positive (see kodachrome). The primary antibody used was rabbit polyclonal antisera generated against a bacterially synthesized protein that corresponds to exon 4 of RhCMV IE1.

Contributor's Morphologic Diagnosis: Lung: Vasculitis, necrotizing, diffuse, severe, non-suppurative, with inclusion-bearing cytomegalic cells and adventitial edema.

Interstitial pneumonia (primarily perivascular in orientation) with intraalveolar fibrin and edema and inclusion-bearing cytomegalic cells.

Contributor's Comment: Diffusely throughout the lung, the adventitia of both arteries and veins is severely edematous and there is multifocal medial necrosis, moderate numbers of intramural inflammatory infiltrates, and swollen endothelial cells (cytomegalic cells). The cytomegalic cells are often sloughed into the lumen, and many contain large magenta intranuclear inclusions. Other cytomegalic cells, most of which appear to be mesenchymal in origin (i.e., fibroblasts and macrophages) are present in the perivascular spaces, and similar cells can be found in the alveolar walls and alveoli, but none are seen in airway epithelium or mucous glands. Alveoli contain eosinophilic proteinaceous fluid as well as fibrinous exudate, and there are mild to moderate inflammatory infiltrates within alveolar walls.

In addition to lung, several organs including the liver, kidney, spleen, salivary gland, adrenal gland, testis, heart, small intestine and brain contained mildly affected blood vessels lined by swollen, often sloughed inclusion-bearing endothelial cells. Other lesions in this macaque included disassociation of hepatic cords and acute renal tubular necrosis.

Cytomegalovirus is a highly species-specific, opportunistic pathogen known to infect many species including humans and is frequently encountered in immunosuppressed human transplant or HIV/AIDS patients. Disease manifestations in humans can differ depending on the cause of the underlying immunosuppression, i.e. CMV retinitis, frequently seen in HIV/AIDs patients, is rarely a complication in transplant recipients (personal communication, Dr. Jose Montoya, transplant infectious disease specialist, Stanford School of Medicine). The lesions seen in this macaque are most consistent with those seen in human transplant recipients rather than in HIV patients (primarily pneumonia and no retinitis). CMV can produce severe systemic disease in rhesus macaques (*Macaca mulatta*) with SIV and has been reported in chemically immunosuppressed cynomolgus macaques inoculated with varicella-zoster virus. Although inclusions were seen in numerous organs in our macaque, the only real evidence of pathology was in the lung.

Because CMV had not previously caused disease in any other transplant monkeys at this institution, the addition of ATG to the immunosuppressive drug regime in this monkey was considered responsible for activation of latent virus and disease manifestation. This macaque was most likely CMV seropositive prior to surgery, as nearly 100% of adult macaques are subclinically infected.

Although the PCR on plasma and frozen lung was negative, the primers used were to rhCMV DNA, not cynoCMV, and there is the possibility that the sequences may have diverged by a few bases (personal communication Dr. Peter Barry, UC

Davis). However, cross reactivity of the rhCMV polyclonal antibodies for IHC was very strong.

AFIP Diagnoses: Lung: Vasculitis, necrotizing, subacute, diffuse, moderate, with inclusion bearing cytomegalic cells, cynomolgus macaque (*Macaca fascicularis*), nonhuman primate.

Conference Comment: Cytomegalovirus is a betaherpesvirus, which unlike alphaherpesvirus is slowly cytolytic and very host specific. Periodic shedding of the latent virus occurs in a variety of body secretions and results in horizontal transmission. Clinical disease is usually inapparent unless concurrent immunosuppression exists. Meningitis, neuritis, arteritis, orchitis and hepatitis are common sequelae in immunodeficient animals.

Cytomegaloviruses cause enlargement of the nucleus and cytoplasm because rather than releasing enveloped virions into the intercellular spaces, they accumulate within intracellular vacuoles. Intranuclear inclusions are typical. Less frequently, intracytoplasmic inclusions are also seen.

The differential diagnosis discussed in conference included SV40 (*Papovavirus*) and adenovirus, both of which often form large basophilic intranuclear inclusions.

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CASE II – CASE 2 (AFIP 2694946)

Signalment: Adult, female, CD1 VAF, mouse

History: Animal was necropsied at the end of a 2-year carcinogenicity study.

Gross Pathology:
Pancreas: Multiple masses and clear fluid filed cysts, 3 – 5 cm.
Ovaries: Bilateral cysts, 0.5 – 1.0 cm.
Uterus: Enlarged with thickened wall.
Eye: Bilateral corneal opacity.

Laboratory Results: None

Contributor's Morphologic Diagnoses: 1. Pancreas, adenomatoid change, marked, multifocal.
2. Pancreas, atrophy and fibrosis marked, multifocal.

Contributor's Comment: The pancreatic mass is composed of multiple cysts, ducts and tubules. Remnants of pancreatic exocrine tissue remain at the perimeter of the mass. Infiltrates of lymphocytes with lesser numbers of neutrophils and lymphoid aggregates are scattered throughout the mass with varying amounts of fibrous tissue. Cysts, ducts and tubules are lined by cuboidal to columnar epithelium, which has abundant eosinophilic cytoplasm with numerous cells having differentiated into goblet cells, this being most notable in duct and tubules. Within the duct and tubular lumens is scattered eosinophilic crystalline material.

Adenomatoid change in mice is considered a non-neoplastic change that can be seen in extrahepatic bile ducts, pancreatic ducts and bronchi. When originating from bile ducts it is reported to be more common in females than males. In this case the origin of the mass was the pancreas.

AFIP Diagnoses: Pancreas: Adenomatoid change, focally extensive, marked, with chronic active pancreatitis and pancreatic atrophy, breed unspecified, mouse.

Conference Comment: The biological behavior of adenomatoid change has not been studied, but it is currently considered to be a non-neoplastic lesion. It is uncommon and unlike other proliferative lesions in rodents (e.g. cholangiofibrosis) it is not associated with chemical exposure.

Conference attendees agreed with the contributor's interpretation of adenomatoid change. While pancreatic carcinoma was considered in the differential diagnosis, it was excluded on the lack of atypia, low mitotic rate, and absence of infiltration.

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References: 1. Greaves P: Digestive System *In:* Histopathology of Preclinical Toxicity Studies, pp. 444-445. New York, NY, 1990.
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CASE III – X5828 (AFIP 2787858)

Signalment: 3-month-old, male, MRL/MpJ-Tnfrsf6-lpr, mouse, (*Mus musculus*)

History: The animal was euthanized at termination of an experimental study

Gross Pathology: Massive generalized lymph node and splenic enlargement, pale kidneys with irregular capsular surface.

Laboratory Results: None

Contributor's Morphologic Diagnosis: 1. Glomerulonephritis, proliferative, diffuse severe.
2. Vasculitis, chronic, disseminated, moderate, multiple organs.
3. Lymphoplasmacytic proliferation/infiltration, disseminated, moderate to severe, multiple organs.

Contributor's Comment: Findings within the kidney included lesions in the glomeruli, tubules, and interstitium:

1. Glomerular proliferation of endothelial and mesangial cells, thickened Bowman's capsules, fibrinoid necrosis of tufts, crescent formation in Bowman's space.
2. Tubular dilation, degeneration, regeneration, and tubular proteinic casts.
3. Interstitial lymphoplasmacytic infiltration and arteritis.

Generalized arterial disease (polyarteritis) with fibrinoid necrosis of variably sized vessels in many organs was accompanied by perivascular lymphoplasmacytic infiltration/proliferation, which was most prominent in the lymph nodes and spleen. Degenerative arteriolar lesions without cellular inflammation also occurred.

MRL/MpJ-Tnfrsf6-lpr (MRL/MpJ-*lpr*/*lpr*) mice homozygous for the *lpr* mutation develop an autoimmune syndrome resembling systemic lupus

erythematosus with massive lymphoproliferation and systemic immune complex disease beginning by eight weeks of age. Although the lesions in the kidney vasculature, particularly glomeruli, are the most prominent, arterial lesions are found in multiple organs as are the lymphoproliferative lesions. The lymphoproliferation is considered to be hyperplastic and not neoplastic as multiple attempts to transplant lymphoid masses to congenic normal recipients have been unsuccessful. Females die at an average age of 17 weeks and males at 22 weeks. The *lpr* mutation is a deletion within the Fas gene. The FAS protein is a cell surface antigen that mediates apoptosis and shows structural homology with several cell surface antigens including tumor necrosis factor. FAS and its' ligand are involved in down-regulating immune reactions.

The lymphoproliferation mutation was found during inbreeding of the MRL/Mp strain derived from crosses among strains LG, AKR, C3H, and C57BL/6. The *lpr* mutation has been introduced into several inbred strains. On different backgrounds it causes varying degrees of lymphoproliferation, autoimmune antibody production, earliness of death, and incidence of severe lupus-like disease. The symptoms can be ameliorated by genetic or monoclonal antibody immune modulation, a restricted calorie diet, or by a fish oil diet that reduces cyclooxygenase metabolites.

AFIP Diagnosis: 1. Multiple organs, vessels: Vasculitis, necrotizing and fibrinoid, chronic active, diffuse, moderate, MRL/MpJ-Tnfrsf6-lpr, mouse (*Mus musculus*).
2. Kidney: Glomerulonephritis, membranoproliferative, segmental to global, diffuse, moderate to severe, with multifocal lymphoplasmacytic interstitial nephritis.
3. Spleen and lymph node: Hyperplasia, lymphoid, diffuse, moderate with mild plasmacytosis.

Conference Comment: The LPR mutation results in deletion of the structural gene for the FAS antigen and failure of lymphocytes to undergo normally programmed cell death. FAS ligand mediated apoptosis is important in the elimination of activated lymphocytes including autoreactive lymphocytes. This dysregulated apoptosis results in increased cell survival and in this case lymphoproliferative disease. The excess population of lymphocytes is largely T-cell and there is enhanced T-helper cell activity. There is polyclonal B-cell production, decreased production of and response to IL-2, and increased production of IL-3. A fulminant humoral autoimmune disease with antinuclear antibodies (ANAs) and immune complex formation resembling Systemic Lupus Erythematosus (SLE) develops.

Circulating antigen-antibody complexes bind to inflammatory cells through their Fc or C3b receptor inducing the release of vasoactive amines increasing vascular permeability. This enhances deposition of immune complexes within small vessel walls. These deposited immune complexes activate neutrophils,

macrophages, and the complement cascade (generalized Type III hypersensitivity). Activated macrophages enhance neutrophil accumulation by secreting TNF-a and IL-1, upregulating endothelial expression of E-selectin and ICAM-1. Complement components, C3a and C5a (anaphylatoxins) and C5a (chemotactic for leukocytes) perpetuate the response. The ensuing vasculitis and tissue damage (most of which neutrophils are responsible for) is the cumulative result of proinflammatory substances, lysosomal enzymes, and free oxygen radical production.

Circulating immune complexes deposit in the glomerular mesangium or form subendothelial deposits. Injury, driven by repeated complex deposition and complement binding, results in chronic immune-complex glomerulonephritis. In this case the glomerulonephritis is characterized by an increase in mesangium and cellularity (membranoproliferative). By immunofluorescence, these immune complexes appear as granular material along the basement membrane, in the mesangium, or both. Nephrotic syndrome (proteinuria, hypoalbuminemia, hypercholesterolemia, and edema) is a common sequela of glomerulonephritis.

Contributor: Department of Genomics and Pathobiology, University of Alabama at Birmingham, Birmingham, AL 35294

References: 1. Festing M: Origins and characteristics of inbred strains of mice. *In*: Genetic Variants of the Laboratory Mouse, eds. Lyon M, Rastan S, Brown S, 3rd ed., vol. 2, pp. 1553-1554. Oxford University Press, Oxford, 1996.
2. National Research Council: Immunodeficient Rodents - A guide to their immunobiology, Husbandry, and Use, pp. 59-62.

CASE IV – 00308 WFUSM (AFIP 2787422)

Signalment: 8-month-old, male, cynomolgus monkey (*Macaca fascicularis*)

History: This animal presented with severe abdominal distension. No other abnormalities were reported. Physical exam and radiographs confirmed fluid in the abdomen. Surgical exploration of the abdomen revealed several hundred milliliters of serosanguineous fluid, hyperemic intestines and nodular, white plaques and fibrinous material lining the body wall and intestines. Euthanasia was elected.

Gross Pathology: A 7-kg male cynomolgus macaque was examined. The animal was in good body condition. No abnormalities were noted externally. Hundreds of white nodules, 2-5mm in size were noted on the serosal surfaces of all abdominal organs and lining the surface of the body wall in the abdominal cavity and diaphragm (see kodachrome), and to a lesser extent the pleural cavity as well. White friable (fibrinous) material was lining the mesentery throughout the small intestines and adhering the tunica vaginalis to the testis. A firm mass, approximately 15 x 5 x 5 cm in size and tan/white in color was found adhered between the greater curvature of the stomach and the colon. Incidental findings included a sutured midline laparotomy incision and an indwelling femoral vein catheter with a small catheter-tip-associated thrombus adherent to the luminal surface of the abdominal vena cava.

Laboratory Results: Virus isolation - positive for simian retrovirus
Serum antibody testing - negative for simian retroviruses

Contributor's Morphologic Diagnosis: Diaphragm (pleural and peritoneal surfaces) - Poorly-differentiated sarcoma; "retroperitoneal fibromatosis"

Contributor's Comment: Retroperitoneal fibromatosis has long been associated with infection with Simian Retrovirus 2. This animal was seronegative for simian retroviruses 1 and 2, but the virus was demonstrated by isolation from mesenteric lymph node. Animals with SRV-associated diseases are often seronegative, making it necessary to screen for the virus by isolation. Retroperitoneal fibromatosis in rhesus macaques has more recently been associated with rhesus rhadinovirus (RRV), a virus similar to the rhadinovirus human herpesvirus 8 (HHV8). A similar rhadinovirus has been identified for cynomolgus macaques, but it is not as well studied. HHV8 is associated with the development of Kaposi's sarcoma in immunosuppressed human beings. RRV encodes a variety of transforming proteins that may contribute to tumorigenesis. Seropositivity for RRV is common in rhesus macaques, and recent work by Mansfield et al. (1999) indicates that RRV infection alone or in combination with SIV does not induce retroperitoneal fibromatosis in short-term studies; therefore SRV infection or a longer passage of time may be required for RRV-associated tumorigenesis. The potential role of rhadinovirus infection in this case was not explored.

AFIP Diagnosis: Diaphragm: Atypical mesenchymal proliferation (Retroperitoneal fibromatosis), cynomolgus monkey (*Macaca fascicularis*).

Conference Comment: Simian Retrovirus 2 (SRV-2) is a member of the family Retroviridae, subfamily Oncovirinae, Type D. It is a spherical RNA virus with a unique 3-layered structure. These viruses may cause immunodeficiency disorders (Simian AIDS) with or without concurrent fibroproliferative lesions. Both

retroperitoneal fibromatosis (RF) and subcutaneous fibrosarcomas are associated with SRV-2 infection. The cell of origin in these proliferative lesions is believed to be a multipotential mesenchymal stem cell.

Retroperitoneal fibromatosis bears histological resemblance to Kaposi sarcoma (KS) in human AIDS patients where human herpesvirus-8 (HHV-8 or KSHV) is thought to be a necessary cofactor for sarcoma development. HHV-8 contains homologs of several genes related to cell proliferation including IL-6 (mitogenic for spindle cells), IL-8 (mitogenic for endothelial cells), bcl-2, and cyclin D.

Rhesus Rhadinoviruses (RRV) (also known as Retroperitoneal fibromatosis-associated herpesvirus of rhesus macaques, RFHVMm) is a gammaherpesvirus that is closely related to HHV-8. Like HHV-8 infection in human AIDS patients with KS formation, a relationship between RRV infections in primate SAIDS patients with RF formation has been suggested. The exact relationship of these newly identified herpesviruses and RF is uncertain and investigation into the possible involvement is being pursued. Complicating the investigation is the fact that up to 90% of normal rhesus macaques are serologically immunoreactive for RRV and remain persistently infected. Although HHV-8 and RRV share structural and functional similarities (ie. both induce increased levels of IL-6), recent research has shown no clear correlation between RRV infection and any specific disease.

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