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Department of Veterinary Pathology
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CONFERENCE 2
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Silver Spring, MD 20910-7500

CASE I – 01-1250-7 (AFIP 2788791)

Signalment: 4-week-old, male, brg +/-, mouse (*Mus musculus*)

History: Two post weanling male mice of this genotype were runted and moribund and euthanasia was requested. Both mice had similar gross and microscopic findings.

Gross Pathology: The gastrointestinal tracts of both mice were diffusely, moderately dilated and had opaque, gray, thickened walls (most noticeable in the proximal small intestine).

Laboratory Results: Serology for a panel of mouse viral and bacterial pathogens was negative.

Contributor's Morphologic Diagnosis: Small intestine: Proliferative enteritis, chronic, segmental to diffuse, severe, lymphoplasmacytic, with marked crypt hyperplasia, villous blunting, and crypt protozoa (*Spironucleus muris*).

Contributor's Comment: Additional findings in these mice consisted of marked proliferative colitis with intralesional spiral bacteria, presumed *Helicobacter spp.,* proliferative gastritis (unknown etiology), severe lymphoid depletion in the spleen and lymph nodes accompanied by marked splenic myeloid hyperplasia and plasmacytosis, lymph node plasmacytosis, and multifocal mild hepatic inflammation.

Microscopic sections are of the proximal small intestine. The mucosa is markedly thickened yet villi are distinctly blunted and occasionally fused. The lamina propria contains an increased density of mononuclear cells, and scattered crypts are dilated and contain luminal pyknotic cell debris. Hyperplastic epithelial cells with increased mitotic figures line most crypts, and crypt lumina are packed with bilaterally symmetric, 12-20 micron trophozoites (*Spironucleus muris*). The trophozoites can also be seen between villi and free in the gut lumen.

Spironucleus muris is an opportunistic protozoal pathogen found predominantly in the upper GI tract of mice, rats and hamsters. *S. muris* should be easy to distinguish from *Giardia muris* based on its size, shape and location (in crypts vs. attached to brush border). *S. muris* rarely causes disease unless the host is otherwise immunocompromised, and susceptibility is age dependent, with 3-week-old mice the most likely to manifest disease. When overgrowth occurs, affected mice are runted, lethargic, have rough hair coats and swollen abdomens. The proximal duodenum may be thickened or contain yellow pasty or foamy ingesta. Mice infected with mouse hepatitis virus can have concurrent GI lesions from both pathogens, and T cell deficient nude (*nu/nu*) mice develop persistent infections, overt signs of disease, and chronically shed cysts in feces.

Brg is a subunit of the BAF complex involved in T cell signaling. Deletion of *brg* results in embryonic lethality and complete lack of thymic development. This mouse was heterozygous for the gene, which resulted in a profound T cell deficient phenotype and increased susceptibility to infection with *S. muris*. Proliferative colitis associated with *Helicobacter spp.* and proliferative gastritis of unknown etiology (personal communication with Dr. Steve Barthold), were other lesions seen in this mouse and represent common entities found in mice with genetically induced or spontaneous immunodeficiencies.

AFIP Diagnosis: 1. Small intestine: Enteritis, lymphoplasmacytic, diffuse, moderate, with crypt hyperplasia, villus blunting and fusion, and myriad trophozoites, etiology consistent with *Spironucleus muris*, brg +/- mouse, rodent. 2. Liver: Hepatitis, subacute, multifocal, mild to moderate.

Conference Comment: *Spironucleus muris* is a member of the *Hexamitidae* family, has a worldwide distribution, and reproduces by longitudinal binary fission. Transmission is direct through ingestion of infective, environmentally resistant, cysts in contaminated food, water, or bedding. The severity of lesions is age, species, and immune status dependent. Trophozoites reside in the intervillar spaces and crypts. Most often they do not attach or invade the mucosa, although it is occasionally reported.

The severity of intestinal lesions in this case, as well as the abundance of organisms, suggests a coinfection (i.e. *Giardia*, mouse hepatitis virus), environmental stressor, or underlying immunodeficiency. In this case, deletion of Brg blocks the normal thymic development of T lymphocytes before the expression of CD4 and CD8 molecules.

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CASE II – CASE 1 (AFIP 2694941)

Signalment: 12-week-old, male, Sprague-Dawley CD VAF, rat

History: Animal was necropsied at the end of a 28 day toxicity study.

Gross Pathology: The liver had multifocal pale areas.

Laboratory Results: None

Contributor's Morphologic Diagnoses: 1. Liver, cholangiofibrosis, marked, multifocal, with oval cell hyperplasia and hepatocellular degeneration.
2. Eosinophilic altered hepatocellular foci.

Contributor's Comment: The liver has an irregular serosal surface with multifocal moderate to marked biliary proliferation and fibrosis. The proliferative ducts extend into and replace portions of the hepatic lobules. Ducts are lined with basophilic epithelium, which varies from attenuated to columnar with piling up of the epithelium in some areas while other portions of ducts are devoid of epithelium giving crescent formations of epithelium. Amounts of epithelial cell cytoplasm are variable and nuclei are prominent, round to oval with open reticulated chromatin pattern. Mitotic figures are moderate in number, 2-5 per high power field. Numerous ducts are dilated and contain eosinophilic material, neutrophils, and necrotic cell debris. The proliferative ducts are surrounded by moderate to marked fibrosis with mixed inflammatory cell infiltrates. Areas of moderate oval cell hyperplasia are scattered throughout the hepatic lobules, as are eosinophilic foci of

hepatocytes and marked hepatocellular degeneration. Other features include mild hemorrhage and hepatocellular necrosis.

In some animals the lesion had progressed to cholangiocellular carcinoma not evident in this specimen.

Cholangiofibrosis is known only to occur in association with chemical exposure. The biological behavior of the lesion depends on the chemical agent, length of exposure and strain of rat. Some cholangiofibrosis are reported to progress to hepatocellular carcinomas.

AFIP Diagnoses: Liver: Cholangiofibrosis, multifocal, marked, with hepatocellular fatty change, Sprague-Dawley rat, rodent.

Conference Comment: Conference participants included cholangiofibrosis and cholangiocarcinoma as their two primary differentials. Features discussed included distribution, degree of invasiveness, cellular characteristics, degree of atypia, and the mitotic rate. Conference participants agreed that the multifocal nature (every portal affected) and the relatively low mitotic rate and degree of cellular atypia were consistent with cholangiofibrosis.

Features of cholangiofibrosis, as listed in the Guides for Toxologic Pathology, include multifocal areas of biliary epithelial hyperplasia with surrounding, often abundant, fibrosis. Proliferating biliary epithelium forms glandular structures that are typically lined by a single layer of cuboidal cells, although multifocal intestinal metaplasia to tall columnar, goblet, or Paneth cells frequently occurs. Glandular structures are mucin producing, crescent shaped, branching, and often ectatic. The edge of lesions may infiltrate the surrounding tissues.

Cholangiocarcinomas present as a mass or nodule, often have a high mitotic rate, cellular atypia, and prominent nuclei. Glandular structures are composed of cuboidal to columnar cells, are lined by one to many cell layers, and have variable mucin production. They may have abundant scirrhous stroma, microinvasion, and evidence of metastasis.

Following the conference, this case was reviewed in consultation with Dr. Jerrold Ward of the National Cancer Institute. Dr. Ward agreed with the contributor and commented that the spectrum of lesions is typical of chemically induced cholangiofibrosis and associated toxic liver lesions.

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CASE III – 01-317 (AFIP 2787753)

Signalment: 3-year-old, male, rhesus macaque (*Macaca mulatta*), nonhuman primate

History: Renal allograft transplantation 298 days ago. Received hu5c8 (anti-CD154/CD40L antibody) and anti-CD25 antibody. Euthanized due to high creatinine and BUN.

Gross Pathology: This young rhesus macaque is in good nutritional condition and weighs 4.33 kg at necropsy. There is a chest and right inguinal tattoo "AK57". The right transplanted allograft kidney weighs 55 grams and measures 6 by 5 by 3 cm. The lung (LLL – left lower lobe or L3) has a hematoma with surrounding white-tan coloring and bilateral white-tan coloring on all lobes.

Laboratory Results: Elevated creatinine and BUN

Contributor's Morphologic Diagnosis: 1. Kidney, arteries and arterioles: Intimal and myointimal proliferation and fibrosis, segmental, diffuse, marked, with multifocal arteritis.
2. Kidney: Nephritis, tubulointerstitial, lymphoplasmacytic and eosinophilic, chronic, diffuse, marked, with tubular loss, interstitial edema, few cellular casts, and mild to moderate transplant glomerulopathy.
3. Ureter: Ureteritis, lymphoplasmacytic and eosinophilic, diffuse, moderate, with submucosal edema.

Contributor's Comment: In human patients, most kidney transplants are lost to slowly evolving, clinically indolent sclerosis in the renal allograft. The renal lesions in this case are consistent with a moderate to high-grade chronic/sclerosing allograft nephropathy (chronic rejection) characterized by occlusive vascular changes, chronic tubulointerstitial nephritis and fibrosis with tubular atrophy/loss, and chronic transplant glomerulopathy. The vascular lesion can result in disruption of the internal elastic membrane; it has been termed fibroproliferative endarteritis, the cardinal morphological feature of chronic allograft rejection in transplanted

organs. In this case, there are lesions in interlobar (hilar), arcuate (corticomedullary), and interlobular (cortical) arteries characterized by elastica disruption or breaks (PAS), myofibroblast proliferation/fibroplasia & fibrosis of the tunica intima/media (Masson's trichrome), and inflammatory cells in the fibrotic intima (arteritis). The vascular lumen is narrowed up 50-60% in a few arteries (grade 3 – severe). The chronic vascular changes are presumed to result from immunological injury or repeated acute vascular rejection episodes; however, hypertension can produce identical changes. Chronic or sclerosing changes can result from ischemia, hypertension, drug effects, infection, increased ureteral pressure, and immune-mediated and nonimmune inflammatory processes.

Additional findings were not coded. Multifocally, the capsule has inflammatory granulomatous foci with anisotropic suture remnants secondary to the transplant surgery. Also, in this slide, note the section of normal ureter. This is the remnant "self" ureter that is ligated and left in the patient coursing alongside the transplanted kidney and ureter. The transplant (donor) ureter is sutured into the recipient's urinary bladder (ureteroneocystostomy) during these pediatric renal transplantations, and undergoes rejection at the same time as the attached donor kidney. Occasionally, the exposed luminal suture tags in the urinary bladder will cause irritation and polypoid mucosal hyperplasia and/or urinary calculi.

The pulmonary vascular lesions are likely the result of pulmonary emboli that lodged in the lung and over time became organized, revascularized thrombi with increased vascular resistance and smooth muscle hypertrophy along with fibrosis (secondary pulmonary hypertension). Pulmonary embolism and thrombo-embolic lesions may be related to the hu5c8 antibody therapy. We have seen multiple chronic transplant rhesus monkeys on hu5c8 therapy with thrombi in the lung and one case in the heart associated with myocardial infarction. The mechanism may involve activated platelets and interaction with endothelial cells. Blood pressure was not measured in this study.

The pulmonary edema is most likely related to barbiturate euthanasia solution, which has been known to cause seepage of proteinaceous fluid into alveoli. The lymphoplasmacytic gastritis is often associated with *Helicobacter pylori* infection in macaques. Other lesions coded above are considered clinicopathologically insignificant.

AFIP Diagnoses: Kidney: Nephritis, tubulointerstitial, lymphoplasmacytic, chronic, diffuse, marked, with fibroproliferative arteritis, lymphoplasmacytic and eosinophilic ureteritis, and mild mesangioproliferative glomerulonephritis, rhesus macaque (*Macaca mulatta*), nonhuman primate

Conference Comment: Hallmarks of chronic allograft nephropathy include interstitial fibrosis, tubular atrophy, fibroproliferative endarteritis (diagnostic for chronic renal

rejection), and glomerular changes. The assortment of changes associated with the glomeruli are included under the term "chronic transplant glomerulopathy". These include subendothelial widening, mesangial expansion, swollen endothelial cells, synechia, and crescent formation. The eventual destruction of the transplant organ in chronic rejection is due to direct alloimmune cytotoxic injury and progressive ischemic injury secondary to the fibroproliferative endarteritis.

In contrast, acute renal rejection is characterized by intimal arteritis and tubulitis. Intimal arteritis is considered highly specific for acute rejection. Less specific changes in the acute rejection include glomerulitis and an interstitial nephritis. Treatment with anti-CD154 antibodies significantly delays the normal progression of lesions associated with graft rejection. Inhibition of CD154 (CD40L) binding to the CD40 receptor on endothelial cells and antigen presenting cells disrupts the usual production of pro-inflammatory and chemotactic factors (ie. IL-1B, TNF-a, IL-12). In addition, the expression of MHC-II molecules, adhesion molecules, and co-stimulatory ligands (B7-1) on endothelial cells and antigen presenting cells is decreased. The cumulative effect is a decrease in mononuclear inflammation and fibrosis and increased graft survival.

Contributor: Walter Reed Army Institute of Research / Naval Medical Research Center, Pathology Departments and the NIDDK/Navy Transplantation and Autoimmunity Branch, Silver Spring, MD, 20910-7500

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CASE IV – 779 (AFIP 2789824)

Signalment: 10-week-old, Leghorn, male, chicken (*Gallus gallus*), avian

History: This chicken was among a group of 8 six-week-old SPF chickens experimentally infected by aerosolization with virulent *Mycoplasma gallisepticum* strain AP3AS. Chickens were kept in isolators prior to and after challenge. Clinical signs consisted of respiratory rales, nasal mucous exudates, and foamy conjunctival exudates. Two weeks after challenge, the bird was euthanized and subjected to postmortem examination.

Gross Pathology: At necropsy, excess mucous exudate was found in nasal cavities and infraorbital sinuses. Tracheal mucosa was thickened throughout the total length of the organ. Areas of consolidation were found at the posterior extremity of the lungs. Right and left abdominal airsacs were severely thickened and contained yellowish muco-caseous exudates.

Laboratory Results: Rapid serum agglutination assay revealed the presence of *Mycoplasma gallisepticum* antibodies in serum collected prior to post mortem. *M. gallisepticum* was also isolated from upper, middle, and lower trachea as well as airsacs.

Contributor's Morphologic Diagnosis: 1. Tracheobronchitis, severe, chronic, lymphoproliferative
2. Pneumonia, localized, chronic, necrogranulomatous
3. Airsacculitis, severe, lymphoproliferative, caseous; *Mycoplasma gallisepticum*

Histologic examination of the trachea revealed a moderate to severe infiltration of the lamina propria with macrophages, lymphocytes, plasma cells, and some heterophils. Also, metaplasia and loss of cilia of epithelial cells were evident in some places.

Histologic examination of the lung showed metaplasia and hyperplasia of the epithelial lining of air capillaries. There is infiltration of macrophages, lymphocytes, plasma cells, and heterophils within the interstitia and in the lamina propria of secondary bronchi. In addition, bronchiectasis and areas of caseous necrosis and granuloma formation are evident.

Airsac lesions were characterized by metaplasia of the epithelial lining, edema, fibroplasia, caseous exudation, lymphocytic hyperplasia, and diffuse and focal mononuclear and heterophil infiltration.

Contributor's Comment: *Mycoplasma gallisepticum* is a poultry pathogen causing chronic respiratory disease and loss of egg production. It is a ubiquitous organism and has been isolated from several avian species including chickens, turkeys, pheasants, quail, and parrots.

M. gallisepticum is one of the smallest self-replicating organisms known, lacks a cell wall, and is unable to survive for long periods outside its natural host. Despite the simplicity of the cellular structure, the molecular mechanisms of pathogenesis and chronicity of *M. gallisepticum* infection are not fully understood. It is postulated that the close relationship between *M. gallisepticum* and its host is reflected in its membrane proteins, the main product of their small genome.

M. gallisepticum expresses a family of phase-variable major membrane proteins, pMGAs, which are responsible for adherence to host cells and are major targets of the host's immune response. Recent *in vivo* studies have revealed that antigenic variation in expression of pMGAs are common, obligating events for successful colonization of the host. PMGA-specific antibodies can operate the switch in pMGA phenotype suggesting that pMGA phase variation is a strategy to avoid the host immune system and to cause chronic infection.

Other mechanisms suggested to contribute to *M. gallisepticum* pathogenicity include ciliostasis, production of inflammatory substances, and direct cytopathic effects by secretion of phospholipases, nucleases, and hydrogen peroxide.

AFIP Diagnosis: 1. Lung: Bronchopneumonia, heterophilic and granulomatous, focally extensive, severe, with lymphofollicular bronchitis, Leghorn chicken (*Gallus gallus*), avian.
2. Airsac: Airsacculitis, heterophilic, diffuse, severe, with multifocal lymphoid proliferation.
3. Trachea: Tracheitis, lymphoplasmacytic and histiocytic, diffuse, moderate, with multifocal epithelial hyperplasia.

Conference Comment: *Mycoplasma gallisepticum* is a member of the order Mycoplasmatales, the smallest self-replicating procaryotes. There are 22 avian mycoplasmas, four of which are considered pathogenic for domestic poultry. The clinical condition is commonly known as Chronic Respiratory Disease (CRD).

Host response typically includes serofibrinous inflammation and activation of the cell mediated response. Many Mycoplasmatales excrete a mutagenic substance causing transformation of host lymphocytes. The result is a proliferation of immature lymphocytes in lymph follicles with invasion into the adjacent tissues. Complicating factors often include coinfection with Newcastle disease virus (Paramyxoviridae), Infectious bronchitis virus (Coronaviridae), or *Escherichia coli*.

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