Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2019-2020

C o n f e r e n c e 15

22 January 2020

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CASE I: 16N131-1 (JPC 4128009).

Signalment: 3.5mo old, female, NOD.*Cg-Prkdc*^{scid}*Il2rg*^{tm1Wjl}/SzJ (NOD-SCID-gamma/NSG) mouse (*Mus musculus*)

History: This mouse was xenografted in the mammary fat pad at 6 weeks of age with tumor cells from a breast cancer patient. The mouse presented moribund, hunched and scruffy two months later, and was subsequently euthanized.

Gross Pathology: The xenograft tumor was not observed at gross necropsy. The mouse was in poor body condition, with marked depletion of external and internal adipose stores. The lungs were mottled dark red, and the spleen was dark red to black and smaller than normal. The small intestine contained small amounts of mucous, and few fecal pellets were present in the descending colon.

Laboratory results: N/A.

Microscopic Description: In sections of brain, there was a severe inflammatory infiltrate composed exclusively of mature and degenerate neutrophils within the third and lateral ventricles, extending into the subjacent neuropil of the hippocampus and cerebrum with associated fragmentation and rarefaction of the neuropil. Several colonies of short rod-shaped bacteria with peripheral clearing were observed within areas of necrosis. The meninges were expanded with a mild inflammatory infiltrate composed predominantly of neutrophils. In sections of lung, multiple arteries contained variably sized accumulations of fibrin, neutrophils, and fewer macrophages, some containing similar rod-shaped bacteria. The perivascular interstitium and alveolar walls were multifocally thickened with few neutrophils and macrophages, some of which contained intracytoplasmic bacteria. In sections of liver, there were few small inflammatory foci composed of degenerate neutrophils associated with hepatocellular

Brain, NOD SCID mouse: Multiple sections of the brain are submitted with cerebellum and brainstem (top left), diencephalon (middle) and telencephalon (inverted at bottom right). The lateral ventricles (black arrows), third ventricle (blue arrow) and fourth ventricle (green arrow) are distended and contain a cellular exudate. (HE, 5X).

necrosis and rod-shaped bacteria, and there were increased numbers of inflammatory cell infiltrates within portal areas, composed primarily of lymphocytes, macrophages, and fewer neutrophils. Occasionally rod-shaped bacteria were observed in Kupffer cells. In sections of bone marrow, there was diffuse and marked myeloid hyperplasia. Hucker-Twort gram stain on sections of brain revealed gram negative rod-shaped bacteria.

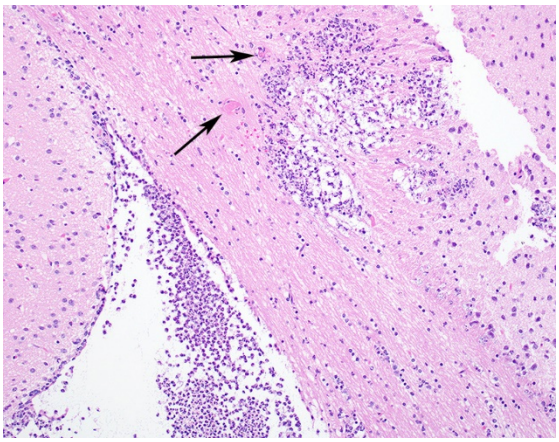
Contributor's Morphologic Diagnosis:
1. Brain: Meningoencephalitis, suppurative, locally extensive, severe, with rod-shaped bacterial colonies.

2. Lung: Pneumonia, suppurative and embolic, multifocal, moderate, with rod-shaped bacterial colonies.

3. Liver: Hepatitis, suppurative and embolic, multifocal, minimal, with rod-shaped bacterial colonies.

4. Bone marrow: Myeloid hyperplasia, diffuse, marked.

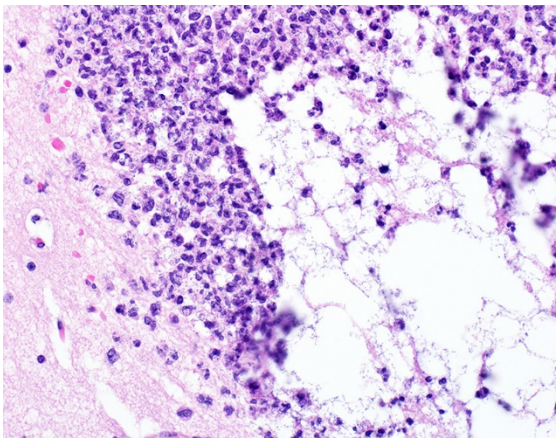
Contributor's Comment: *Klebsiella spp*. are gram-negative rod-shaped bacteria that are typically commensals in mice, and are not a significant cause of naturally occurring disease. *K. oxytoca* is ubiquitous in the environment, and can be isolated from the gastrointestinal tract, nasopharynx, lung, skin, and mucous membranes of healthy animals and humans.[2,7,14] However, these organisms may become opportunistic pathogens in certain situations, and in both humans and animals infections with *K. pneumoniae* and *oxytoca* are most commonly associated with clinical disease. *Klebsiella oxytoca* can cause suppurative lesions in various organ systems in mice, particularly the reproductive tract, where it has been associated with suppurative endometritis, salpingitis, and perioophortitis often progressing to peritonitis and abscess formation. Other reported opportunistic infections in rats and mice include perianal dermatitis, otitis media, cystitis, pyelonephritis, keratoconjunctivitis, Harderian gland adenitis, oral infection, subcutaneous, abdominal and hepatic abscesses, pneumonia, meningitis, endotoxemia and septicemia. [1,3,8,14] In humans, *Klebsiella oxytoca* and *pneumoniae* are considered important causes of nosocomial infections in hospitalized patients, and has been implicated in community acquired pneumonia, adult and



Brain, NOD SCID mouse: Neutrophils occupy the lateral ventricle and infiltrate the adjacent parenchyma, resulting in cavitation and thrombosis of parenchymal capillaries (arrows). (HE, 200X). (Photo courtesy of: In Vivo Animal Core, Unit for Laboratory Animal Medicine, University of Michigan Medical School, Ann Arbor, MI 48109 http://animalcare.umich.edu/business-services/vivo-animal-core)

neonatal sepsis and bacteremia, septic arthritis, soft tissue abscesses, and urinary tract infections, and chronic nasal infections.[1,2,10] *K. oxytoca* is also suspected to be the etiologic agent responsible for antibiotic-associated hemorrhagic colitis (AAHC) in humans, which occurs following antibiotic therapy and is characterized by bloody diarrhea, abdominal cramping and segmental hemorrhagic typhlocolitis [1,2,14]. Antibiotic therapy has been shown to promote abnormal colonization by *Klebsiella spp.* in humans[1] and rodents,[5] and an animal model of AAHC has been developed using oral administration of antibiotics in rats followed by oral infection with *K. oxytoca*.[6]

Immune status of the host plays an integral role in the pathogenesis of disease by *Klebsiella spp*. Certain strains and genetically modified mouse lines are more prone to developing lesions associated with *Klebsiella spp*. infection. Suppurative otitis media, urogenital tract infections, and pneumonia have been reported in substrains of C3H/HeJ mice and NMRI- *Foxn1*^{nu} mice, and LWE.1AR1 rats, and chronic renal inflammatory lesions and ascending urinary tract infections in NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ (NSG) mice. [1,2,4,7,14] C3H/HeJ substrains are hyporesponsive to lipopolysaccharide (LPS) produced by gram-negative bacteria, due to a single amino acid substitution in the toll-like receptor 4 (TLR4) protein, NMRI- *Foxn1*^{nu} (nude) mice lack a thymus and are therefore T-cell deficient, and LEW.1AR-*iddm* rats are at risk for infection secondary to development of diabetes mellitus. Finally, NSG mice have multiple mutations including the *Prkdc* severe combined immune deficiency (scid) mutation, IL2 gamma deficiency, mutation of the C5 complement gene, and a novel MHC haplotype which leads to lack of normal T

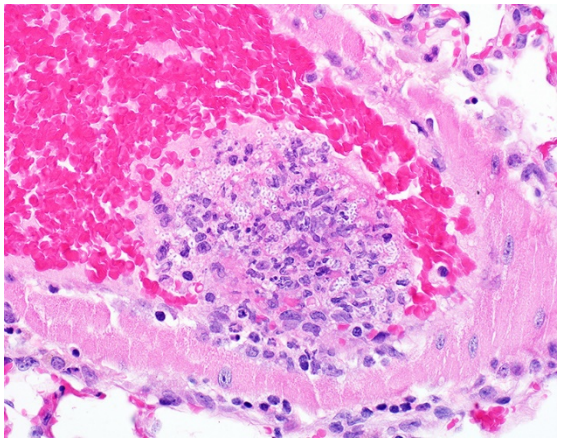


Brain, NOD SCID mouse: Neutrophils occupy the lateral ventricle and infiltrate the adjacent parenchyma, resulting in cavitation and thrombosis of parenchymal capillaries (arrows). (HE, 200X). (Photo courtesy of: In Vivo Animal Core, Unit for Laboratory Animal Medicine, University of Michigan Medical School, Ann Arbor, MI 48109 http://animalcare.umich.edu/business-services/vivo-animal-core)

and B lymphocytes and NK cells, and deficient cytokine and complement signaling and function.[4] Increasing age may also play a contributory role in pathogenesis of *Klebsiella* infection, as suppurative reproductive lesions due to *K. oxytoca* infection have been reported in National Toxicology Program (NTP) chronic chemical carcinogenesis bioassays.[3]

Klebsiella exerts its pathogenic effects by taking advantage of an immunosuppressed or immunocompromised host, and through the use of several virulence factors. The presence of a distinct polysaccharide capsule observed as a peripheral clearing on routine light microscopy is correlated with virulence of the organism, enabling it to resist phagocytosis and bactericidal components of serum.[1,7,10] In addition, several in vitro studies in humans and in mice have shown that *K. oxytoca* produces a cytotoxin, tilivalline, which induces cell death through inhibition of DNA synthesis.[2,14] Furthermore, genomic studies on tilivalline-producing *K. oxytoca* in both humans and mice revealed a number of genes associated with virulence potential.[2] Finally, a substrain of *K. oxytoca* (TNM3) has been shown to produce an immunosuppressive polysaccharide, AZ9, which is associated with decreased IL4 and IFNg responses leading to an overall depressed Th2-type immune response.[11,12]

In the present case, lesions were clearly representative of a septicemic process with multiple suppurative and embolic lesions in several organs including the lung, liver, and brain, and a myeloid response in the bone marrow. By light microscopy and gram staining, the observed peripheral clearing around the gram-negative, rod-shaped bacterial organisms is characteristic of *Klebsiella spp*. The affected animal in this case was a NSG mouse, which as previously discussed is a severely immunodeficient mouse model prone to opportunistic infection. These mice are commonly used to model development, progression, and metastasis of human tumor cells in vivo, through xenotransplantation experiments.



Lung, NOD SCID mouse: Septic fibrinocellular thrombi are present within the lumen of pulmonary arterioles. Incorporated macrophages and neutrophils often contain encapsulated bacilli within their cytoplasm. (HE, 400X). (Photo courtesy of: In Vivo Animal Core, Unit for Laboratory Animal Medicine, University of Michigan Medical School, Ann Arbor, MI 48109 http://animalcare.umich.edu/business-services/vivo-

The source of the infection is unknown, but it is possible that *K. oxytoca* was introduced through human contact as this organism can be spread from humans to rodents,[1] or through environmental contamination. One major concern with the presence of such opportunistic organisms in animals maintained with a well-defined hygienic status (specific pathogen-free) is that they are becoming more common and relevant as causes of disease in such colonies,[1] so

knowledge of these organisms is imperative to maintaining high standards of animal welfare and research quality. Furthermore, in experiments utilizing immunocompromised mice for serial tumor passage or xenograftment, transfer of tumor cells or other biologic material contaminated with infectious agents such as *Klebsiella* is a potential significant risk factor to the health recipient mice, as well as the quality of the experimental outcome due to morbidity and mortality secondary to opportunistic, and unexpected, infection.

Contributing Institution:

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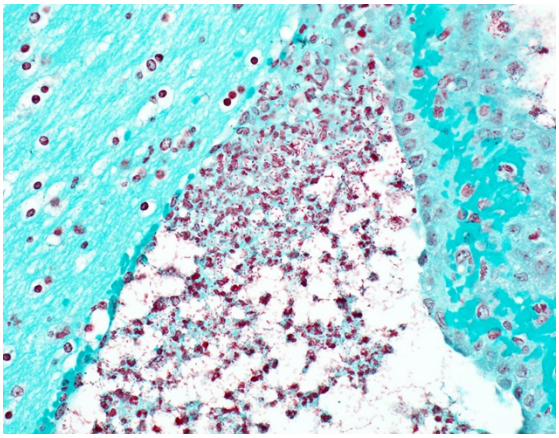

Cerebrum, NOD SCID mouse. Numerous discrete encapsulated gram-negative bacilli are present within the ventricular exudate. (Hucker-Twort, 400X) (Photo courtesy of: In Vivo Animal Core, Unit for Laboratory Animal Medicine, University of Michigan Medical School, Ann Arbor, MI 48109 http://animalcare.umich.edu/business-services/vivo-animal-core)

JPC Diagnosis: Brain: Ventriculitis, periventriculitis, and meningitis, necrotizing and suppurative, multifocal to coalescing, severe, with vasculitis, thrombosis gliosis, and numerous bacilli.

JPC Comment: The contributor has provided an outstanding and thorough review of this opportunistic infection in laboratory rodents, especially immunosuppressed models.

Tilvallin, as mentioned bythe contributor, is a gene product of *K. oxytoca* which induces apoptosis and loss of barrier integrity *in vitro* in human epithelial cells, which suggests a possible pathogenesis inhuman antibiotic-associated hemorrhagic colitis (AAHC).[12] Further investigation of the biosynthesis of this compound has demonstrated a number of other secondary metabolites, also pyrrolobenzodiazpines (PBD), including tilmysin and culdesacin. The combination of tilmysin with indole actually yields tilvallin. The PBD family of compounds are potent cytotoxic agents which demonstrate both antibacterial and anticancer activity due to DNA alkylation and formation of PBD-DNA adducts. In addition, tilvallin is also a microtubule stabilizing agent, a class of compunds that shift the balance of cellular tubulin from soluble to polymerized and are widely used as anticancer agents. This class also includes taxol, a widely used chemotherapeutic[12].

In 2011, the *Enterobacteriaceae* genus *Raoultella* (named after the French bacteriologist Didier Raoult) was separated from Klebsiella through the use of molecular techniques, as well as the identification of

growth at 10^{o} C and use of L-sorbose as a carbon source). This genus include four species: *Raoultella ornithinolytica, R. planticola, R. terrigena, and R. electrica.*[9] These bacilli are found in plants and soli in aquatic environments. R. ornithinolytica and R. planticola are considered emerging human pathogen, which results in biliary tract infections in elderly or immunosuppressed patients with malignancies or who have undergone invasive procedures. It is considered likely that a number of *Klebsiella* infections diagnosed historically may actually be of bacilli of this genus.[9]

The moderator discussed *Rodentibacter pneumotropica* as a common opportunist in immunosuppressed mice (which is usually difficult to see on HE and special stains), which highlights that the name of this common opportunist has changed within the last few years (for those who are trying to keep up with the microbiologists. The moderator also commented on the description of a "smaller than normal" spleen, as the normal weight of the spleen of an immunocompent mouse is 0.2g, and of a NOD-SCID mouse is 0.02g, highlighting not only the size difference of spleens, but the need to use absolute weights in this species. As this was a xenograft animal, the moderator wondered about the possibility of irradiation prior to engraftment in this individual further complicating this picture. Unfortunately lab data was not available in this case to distinguish between *K. oxtyoca* and *K. pneumoniae* in this case, so the moderator was loathe to pick one species over the other.

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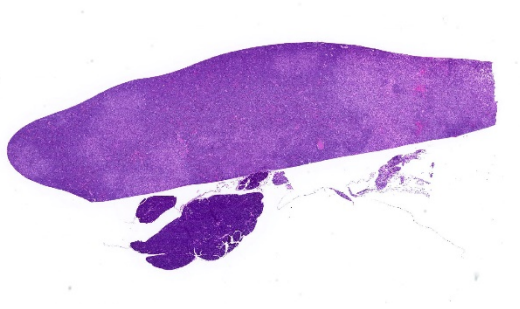
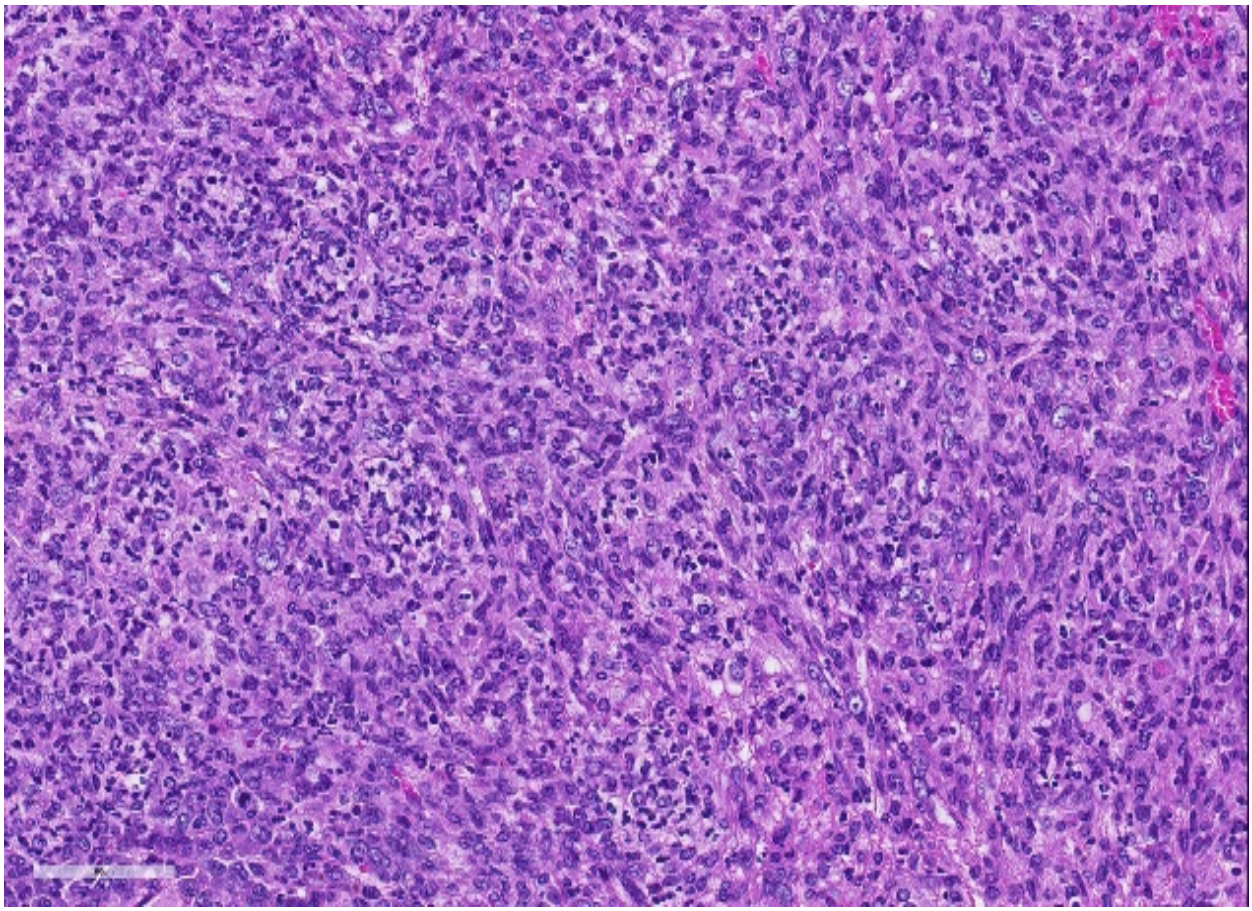
CASE II: MS18-3968 (JPC 4136806).

Signalment: Adult, male, albino, CYBB[ko] mouse, Mus musculus

History: NSG.Cybb[KO] mouse observed with scruffy hair coat, slightly hunched posture and pale ears. Mice of this strain have been spontaneously dying. These mice are on corn cob bedding and are provided with TMS antibiotic water. The feed is regular rodent chow and is autoclaved with the cage setup. The antibiotic water is made by sterilizing tap water in the autoclave and then adding TMS at the room in a Biological Safety Cabinet (BSC). The cages are sterile when they arrive at the room with aseptic technique used to transfer the mice from the dirty cage to the clean cage. All manipulation is performed in a BSC.



Spleen and pancreas, CYBB[ko] mouse. A section of spleen, pancreas, and mesentery is submitted. The spleen is 4-5 times normal thickness and normal follicular and sinusoidal architecture is not evident. (HE, 5X)



Spleen, CYBB[ko] mouse. Effacing approximately 50% of the splenic architecture in a nodular pattern are large numbers of macrophages (often spindling), admixed with large numbers of neutrophils. Mitotic figures are common. (HE, 400X)

Gross Pathology: Presented for necropsy was a live adult male white mouse. The animal appeared to be in good nutritional condition. The animal had a roughened hair coat, yet was alert and active in the transport box. The animal was euthanized with CO2.

Upon opening the carcass, adequate adipose stores were observed. The subcutis was slightly tacky indicating mild dehydration. Upon opening the peritoneal cavity, the spleen was enlarged approximately three times normal size and contained multifocal to coalescing pale tan masses that expanded above the capsular surface. The liver was also enlarged approximately three times normal size with multifocal to coalescing pale tan slightly raised masses scattered throughout.

Upon opening the pleural cavity, the lungs contained numerous light red pinpoint scattered foci. There was a 1 mm in diameter clear cyst in the right caudal lung lobe.

Lesions were not observed in the brain, heart, kidneys, pancreas, the entire male reproductive tract, and the entire gastrointestinal tract that has scant ingesta/digesta throughout and multiple formed feces in the descending colon.

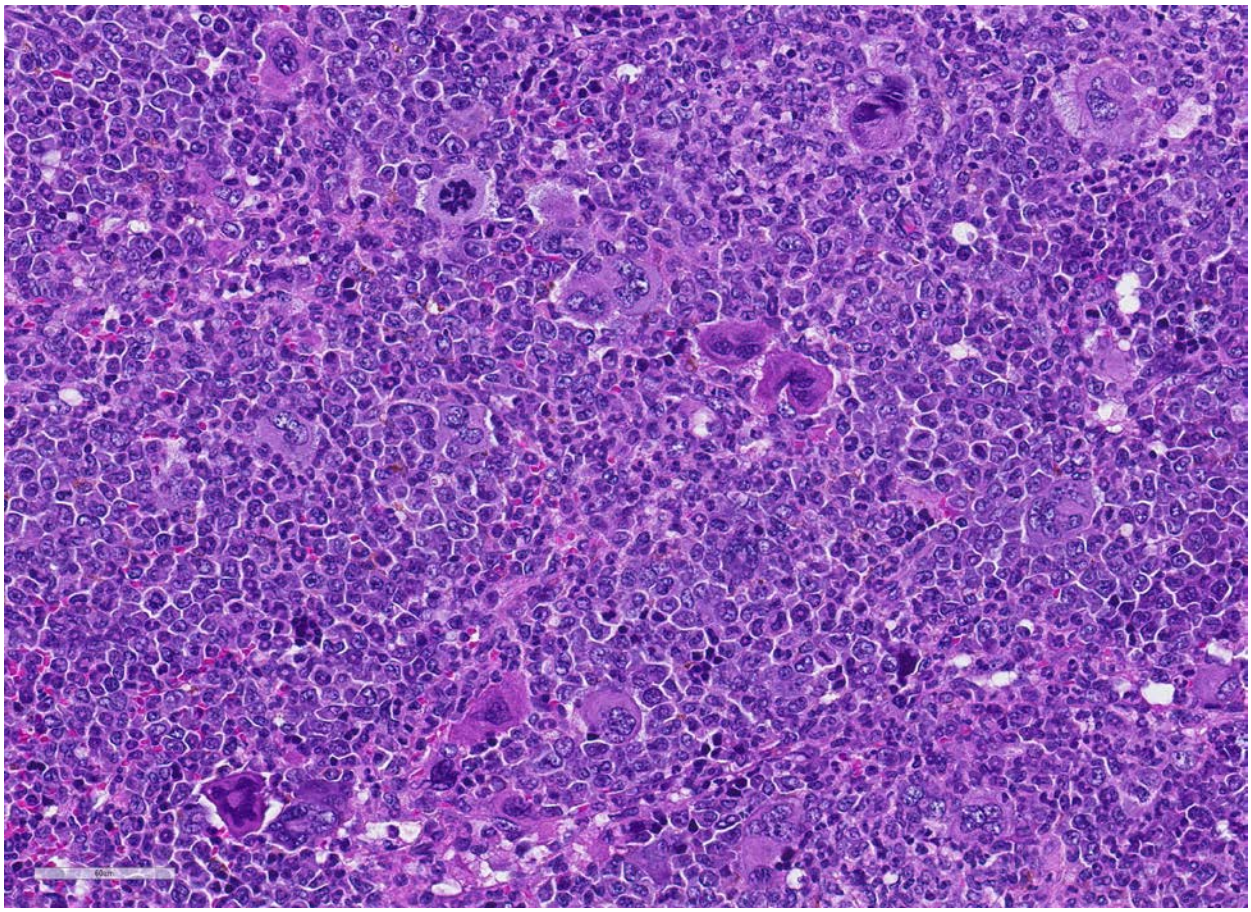
Laboratory results: Microbiology yielded *Candida parapsilosis* from the liver.

Microscopic Description: Spleen – Multifocal to coalescing granulomas with epitheliod macrophages, many with neutrophils, and a coarse fibrous connective tissue network are observed. In a number of these are intracellular, one to a few, 1-5 um round to oval yeast organisms with a clear halo, and which a number are seen budding on PAS stain. The adjacent parenchyma has a moderate granulocytic hyperplasia. Similar lesions were observed in the liver, and yeast were observed in the liver, kidneys (tubulitis) and lungs (alveolitis).

Contributor's Morphologic Diagnosis:

Spleen, splenitis, pyogranulomatous and fibrosing, multifocal to coalescing, severe, chronic with intralesional yeast, *Candida parapsilosis*

Contributor's Comment:
NSG.Cybb{KO} mice are a genetically altered mouse strain that is severely immunocompromised. NSG (NOD.Cg-Prkd^{scid} Il2rg$^{tm1Wjl/}$SzJ) is a lymphocyte deficient strain, and Cybb KO (CYBB gene is located on X-chromosome, and encodes the gp91^{phox} protein) is the most common



Spleen, CYBB[ko] mouse. The splenic red pulp is filled with immature granulocytes with a predominance of band cells (with doughnut-shaped nuclei), and fewer islands of hyperchromatic erythrocyte precursors as well as megakaryocytes. (HE, 400X)

form of Chronic Granulomatous Disease (CGD).[12] CGD is an immunodeficiency with a defect in phagocytes (macrophages, neutrophils) to produce reactive oxygen species (ROS) which are needed for microbicidal activity. Normally, ROS are generated by phagocyte NADPH oxidase. This enzyme is composed of 5 subunits; 2 plasma membranes and 3 cytosolic. Membrane bound are transmembrane glycoproteins (gp), one with 91kD mass called $gp91^{phox}$ (phox for phagocyte oxidase; also known as NOX2[neutrophil oxidase-2]) and a 22kD gp called $p22^{phox}$. These two form a heterodimer. The 3 cytosolic subunits ($p40^{phox}$, $p47^{phox}$, $p67^{phox}$) form a heterotrimer. The genes of the five components are CYBB [cytochrome b beta] located on the X-chromosome encoding $gp91^{phox}$, CYBA [cytochrome b alpha]encoding $p22^{phox}$, NCF1[neutrophil cytosol factor] encoding $p47^{phox}$, NCF2 encoding $p67^{phox}$, and NCF4 encoding $p40^{phox}$.[10]

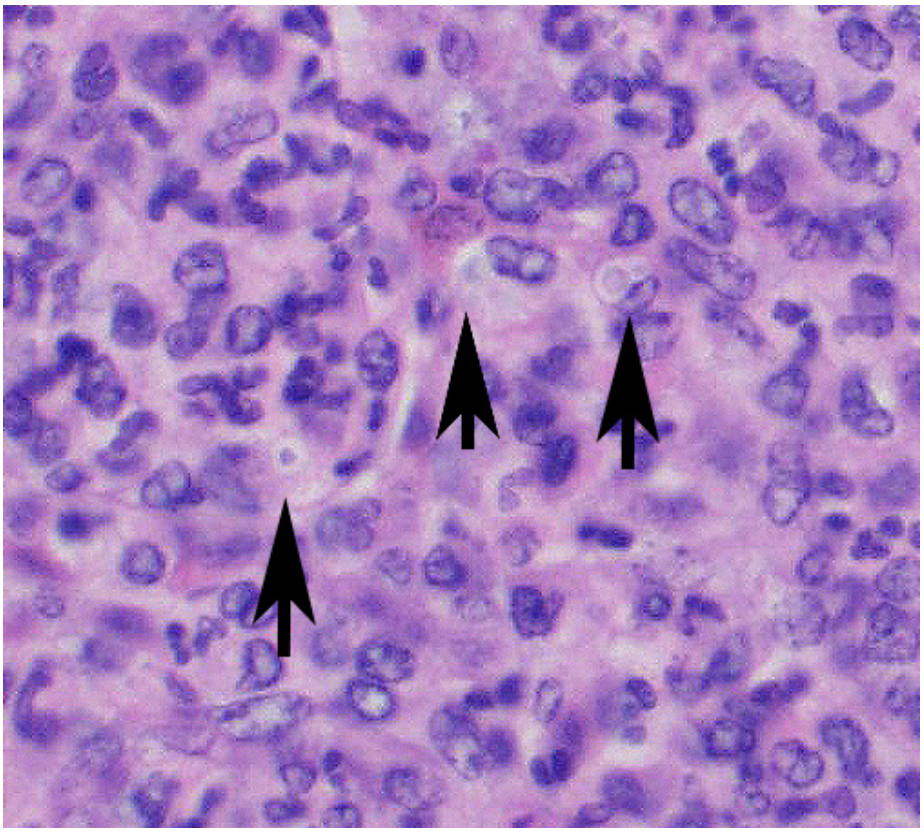
The most common form of CGD involves the CYBB gene (deletion, frameshift, nonsense, missense, splice site mutation) that affects mostly males because of the predominant mode of genetic transmission (X- chromosome).[8] Interestingly, heterozygous mothers of affected males will carry a proportion of innate immune cells that are fully oxidase deficient. These individuals are prone to both infectious complications and autoimmune diseases. Of note, the risk of developing autoimmune disease was not at all related to degree of residual oxidase activity as it is in developing an infection. This suggests that even the presence of a minority of cells with absent oxidase activity predisposes to a dysregulated immune response in a dominant form.[12]

CGD appears to be a dysregulated granulomatous inflammation in various organs including the GI tract, respiratory tract, as well as the eye and urinary tract. In some patients a true autoimmune disease was exhibited; systemic lupus erythematosus, rheumatoid arthritis, inflammatory bowel disease, Sjogren's syndrome and atopic dermatitis. Interestingly, a large percentage of human patients did not show any evidence of infection.[13]

Some patients with CGD suffer from a variety of recurrent bacterial and fungal diseases. The most common bacterial infections include *Staphylococcus aureus, Klebsiella spp., Burkholderia cepacia, Serratia marcescens* and *Salmonella spp.*[9,14] Fungal infections include *Aspergillus fumigatus, A. nidulans, A. niger, A. flavus, Zygomycota* (primarily *Rhizopus spp.), Candida spp., Trichosporon spp., Paecilomyces spp., Scedosporium spp., Penicillium spp., Acremonium spp., Alternaria spp., Inonotus spp., Exophiala, Chrysosporium spp., Fusarium spp., Microascus spp., and Hansela spp.*[7,15] *A. nidulans* seems to have a unique interaction with CGD hosts.[7] *Geosmithia argillacea* has recently been shown to be mistakenly identified *as Paecilomyces spp.* in the past.[6] Dimorphic yeast form infections are exceedingly rare with only one reported case each of *Coccidioides immitis,*[14] *Histoplasma capsulatum,*[14] *and Sporothrix schenckii.*[14]

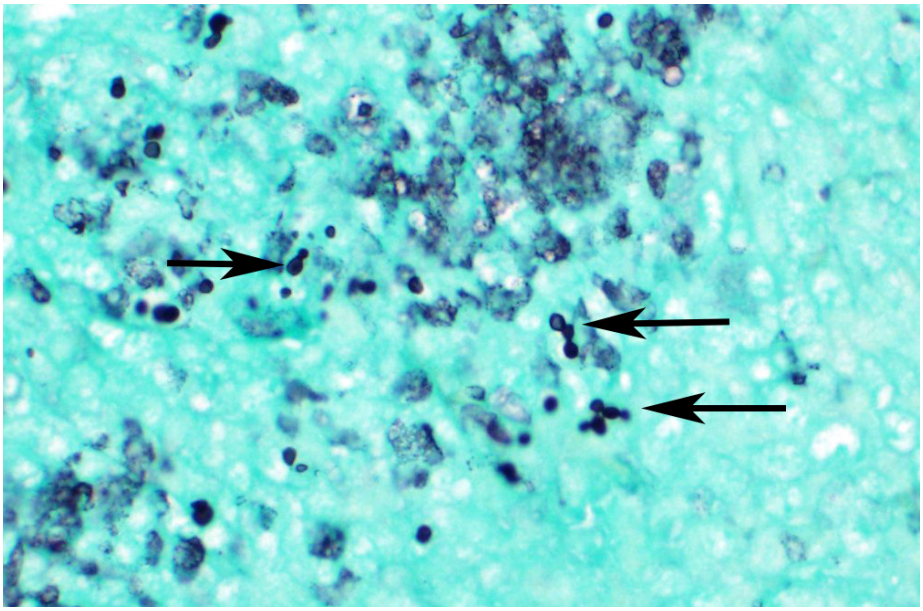
In this case *Candida parapsilosis* was the pathogenic yeast identified. *Candida* species are mainly found in the gastrointestinal tract of humans[9]. *C. parapsilosis* is found frequently on the skin and hands[11]. Although commensal, they can become pathogenic when host defense mechanisms or anatomical barriers are compromised.[10] *C. parapsilosis* is currently the second leading cause of candidemia which is associated with a high morbidity and mortality rate in humans.[12] In CGD humans, *Candida* species were isolated from meningitis, fungemia, lymphadenitis.[13]

C. parapsilosis is actually a complex of 3 distinct genetic species: *C. parapsilosis sensu stricto, C. orthopsilosis* and *C. metapsilosis.*[2,10] Polymorphisms in the genes COX3 (mitochondrial gene cytochrome oxidase subunit 3), SADH (secondary alcohol dehydrogenase) and SYA1 (putative alanyl-tRNA synthetase) distinguish the three species.[5] Virulence of *C. parapsilosis* is related to factors such as cell wall constituents, adhesion to biotic and abiotic structures leading to biofilm formation, and extracellular enzymes such as aspartic proteases, phospholipases and lipases. Aspartic proteases promote tissue colonization and invasion by rupturing host mucosal membranes. They may also aid in dispersion of biofilms. Phospholipases promote rupture of host cell membranes. Lipases help with acquiring nutrition, support fungal growth, mediate adhesion to cells and tissues, and coordinate yeast interactions with enzymes and immune cells during the infectious process.[11]



Spleen, CYBB[ko] mouse. Macrophages contain one or more 3-5um intracytoplasmic yeasts. (HE, 400X)

Initial attachment of *Candida* to host cells is followed by cell division, proliferation (forming yeast and pseudohyphae) and subsequent biofilm formation. Biofilm formation is an important virulence factor as it confers significant resistance to antifungal therapy by



Spleen, CYBB[ko] mouse. A silver stain demonstrates budding yeasts. (GMS, 400X)

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JPC Diagnosis: 1. Spleen: Splenitis, pyogranulomatous, diffuse, severe, with intrahistiocytic yeasts. 2. Spleen, red pulp: Extramedullary hematopoiesis, diffuse, severe.

limiting the penetration of substances through the matrix (water, ions, carbohydrates, proteins and nucleic acids) and protecting fungal cells from host immune responses[14]. Morphologically, *C. parapsilosis* is unable to form true hyphae, but forming pseudohyphae is associated with virulence.[11]

This case is somewhat baffling given that the animal was in a sterile environment with sterile bedding, caging and water yet still was exposed to *Candida*. However, in humans, *C. parapsilosis* is frequently isolated from hands and from sterile body sites. Thus, human transmission may have been possible. Overall, three mice developed similar lesions, with two having recognizable yeast organisms on histopathology. One should be vigilant and be alert for uncommon infections in a laboratory setting.

Contributing Institution:

JPC Comment: The contributor has provided an excellent review of this animal model of an uncommon inherited primary immunodeficiency, the mechanism of the immunodeficiency, as well as a review of Candida sp. in general as well as *C. parasilopsis*, the particular pathogen in this case.

As mentioned by the contributor, chronic granulomatous disease (CGD) is an uncommon X-linked immunodeficiency in humans resulting in frequent bacterial and fiungal infections. Patients with CGD may possess a defect in one of four different structural proteins in NADPH oxidase.[8,10] NADPH oxidase catalyzes the transfer of a single electron from NADPH to molecular oxygen, generating one of four reactive oxygen species, from the superoxide radical, including the peroxynitrite anion, hydroxyl anion, hypochlorous acid, and nitryl chloride. [8,10]

The contributor mentions the many types of fungal infection which are seen in immunsuppressed individuals. The reason for this is that mononuclear phagocyte activity is the major driver of resistance to systemic mycoses. Patients suffering from systemic mycosis have shown consistent benefit from concurrent administration of macrophage colony-stimulating factor. Patients with CGD are treated with continuous antibacterial and antifungal medications, and the only current long-term treatment is allogeneic hematopoitic stem cell transplants.

Interestingly, this WSC conference submission is not the JPC's first encounter with this mutant strain of mouse from the National Institutes of Health, Following a rotation at the Dept. of Veterinary Resources at the NIH, then resident Dr. Shannon Lacy (himself a former resident coodinator of the Wednesday Slide Conference published an article about infection seen in this same strain of knockout mice with another saprophytic fungus, *Trichosporoon beigelli*.[8] Like *Candida, Trichosporoon* is part of the normal flora of human skin and GI tract. This was the first report of disseminated trichosporoonosis in the laboratory mice of any immunosuppressed strain.[8]

The moderator noted that in this case, the yeasts in the submitted slide did not make either pseudohyphae or hyphae, which is consistent with the literature. *C. parapsilosis* apparently makes pseudohyphae in culture and within biofilms and not in tissue. It also does not produce true hyphae.

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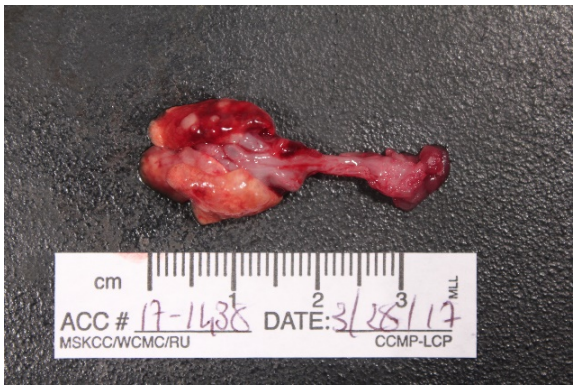
CASE III: 17-1438 1-2 (JPC 4101223)

Signalment: Two-month-old, intact female mouse, *Mus musculus*.

History: The mouse was purchased from a non-conventional vendor (pet shop) and it was enrolled in a study as model of autoimmune colitis. The animal was submitted for euthanasia and necropsy given the loss of body weight (10% in the previous 2 weeks) and hunched posture. No other mouse in the group, from the same source and in the same experimental conditions, exhibited any similar or different sign of disease.

Gross Pathology: The lung lobes are diffusely expanded, firm and mottled (Fig.1). Within the cranial regions there are



Lungs, mouse. There are extensive areas which is most prominent in the left lung. Airways are multifocally markedly ectatic, nodular in appearance and filled with exudate. (Photo courtesy of: Laboratory of Comparative Pathology; Hospital for Special Surgery, Memorial Sloan Kettering Cancer Center, The Rockefeller University, Weill Cornell Medicine. https://www.mskcc.org/research-areas/programs-centers/comparative-medicine-pathology)

multiple grey-white nodules measuring about 0.5 to 1 mm in diameter (consistent with bronchiectasis).

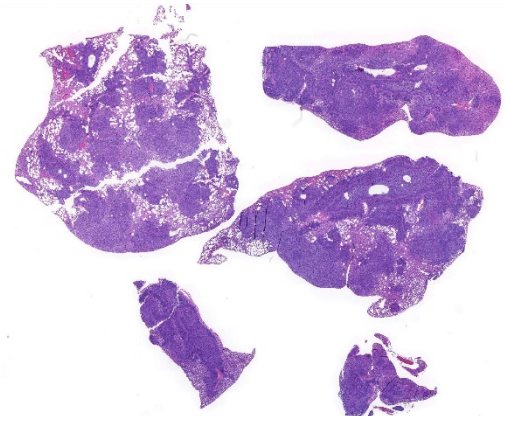
Laboratory results: PCR for Mycoplasma spp. on fresh frozen lung tissue was positive

Microscopic Description: About 80 % of the parenchyma is affected in a multifocal to coalescing fashion by the infiltration and accumulation of a large number of mostly viable and occasionally degenerated neutrophils, admixed with few foamy macrophages, obliterating the lumen of alveoli, bronchioles and bronchi (Fig.2). The adjacent parenchyma is atelectatic. Few bronchial and bronchiolar structures exhibit distorted outlines with dilation (bronchiectasis and bronchiolectasis), thickening of the lining epithelium with piling up of nuclei (hyperplasia), and luminal narrowing by polypoid-like structures, formed by a fibrous stalk and lined by one or more layer of cuboidal to columnar epithelium (bronchiolitis obliterans). Multifocally the respiratory epithelium is replaced by one or more layers of cells with features of squamous epithelium (squamous metaplasia, Fig. 3). Large cuffs composed of lymphocytes and plasmacells surround the intrapulmonary branches of the bronchial tree. Protein rich fluid is multifocally present within few alveoli (alveolar edema).

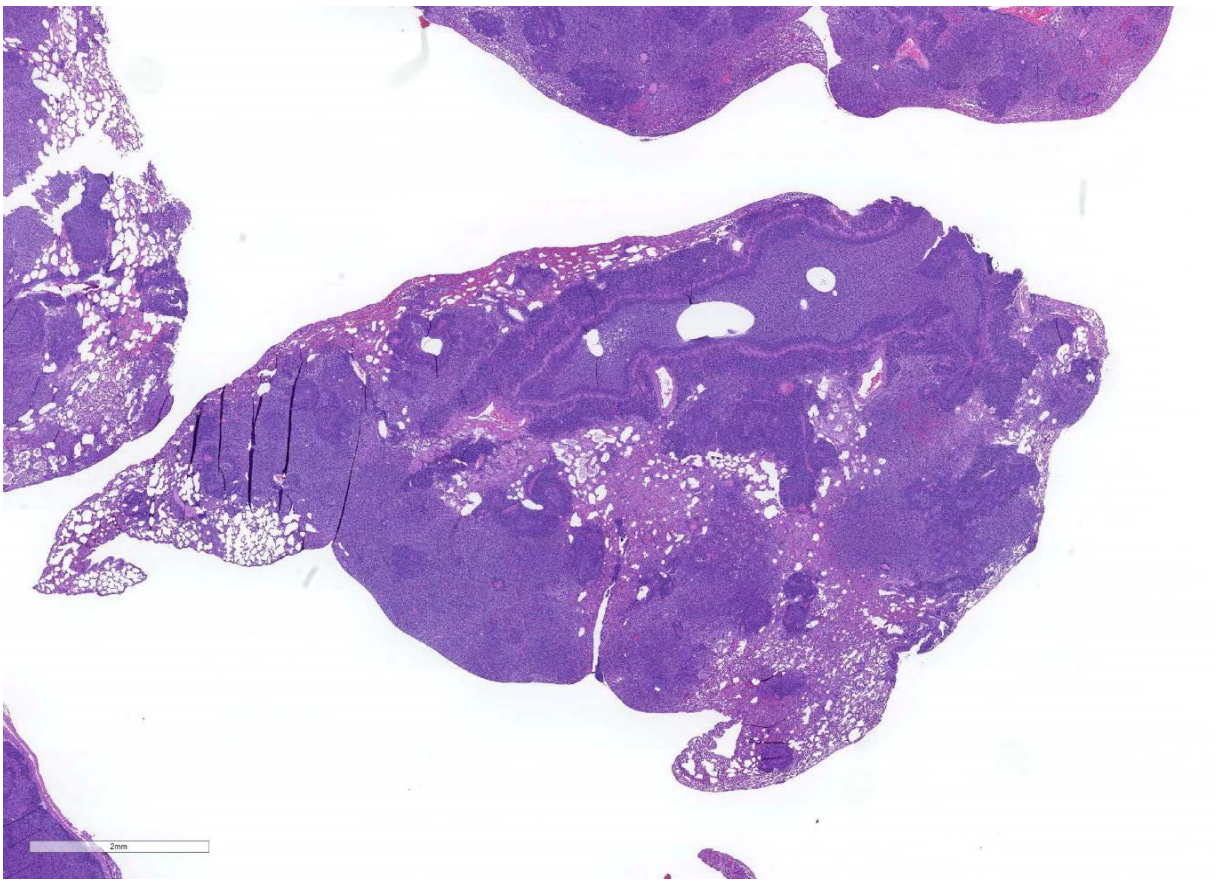
Contributor's Morphologic Diagnosis:

Lungs: suppurative bronchopneumonia, subacute, severe with bronchiectasis, bronchiolectasis, bronchiolitis obliterans and squamous metaplasia, consistent with *M. pulmonis* infection

Contributor's Comment: *Mycoplasma* is a genus of bacteria belonging to the order Mycoplasmatales, family Mycoplasmataceae, class Mollicutes. The class name indicates the lack of a wall around the cell membrane, which is a peculiar feature of these bacteria. As such



Lungs, mouse. Multiple sections of lung are submitted. Inflammatory changes are focused on airways and affect from 80-100% of each section. (HE, 6X)



Lungs, mouse. Multiple sections of lung are submitted. Higher magnification of an affected lobe with a markedly dilated, exudate-filled airway (bronchiectasis). (HE, 15X)

they are classified as gram-indeterminate. Mycoplasmae are divided into 2 clusters: hemotropic and pneumoniae, based on 16S sequencing.

The "pneumoniae" Mycoplasma are often non-pathogenic and are found in the genital and respiratory tracts. Mycoplasma species that are hosted in laboratory mice are: *M. pulmonis*, *M. arthritidis*, *M. neurolyticum*, *M. collis*, *M. muris*, however *M. pulmonis* only is a significant pathogen.

Factors such as Sendai virus infection, high concentration of ammonia in the environment and co-infection with *Pasteurella pneumotropica* play an important role in the clinical onset of the disease in subclinically infected mice. The modern husbandry standards of mouse facilities and health monitoring plans in research institutions contributed to decrease the incidence of mycoplasmosis despite the rather high percentage of infected animals.

Overall, mice are less susceptible than laboratory rats to the disease, however susceptibility to the disease varies upon the mouse strain, with the B6 being resistant and the C3H quite sensitive. Moreover, females seem to develop more severe forms. The natural disease in immunocompetent mice is usually characterized by weight loss, respiratory symptoms and head tilt or vestibular signs in case of concomitant

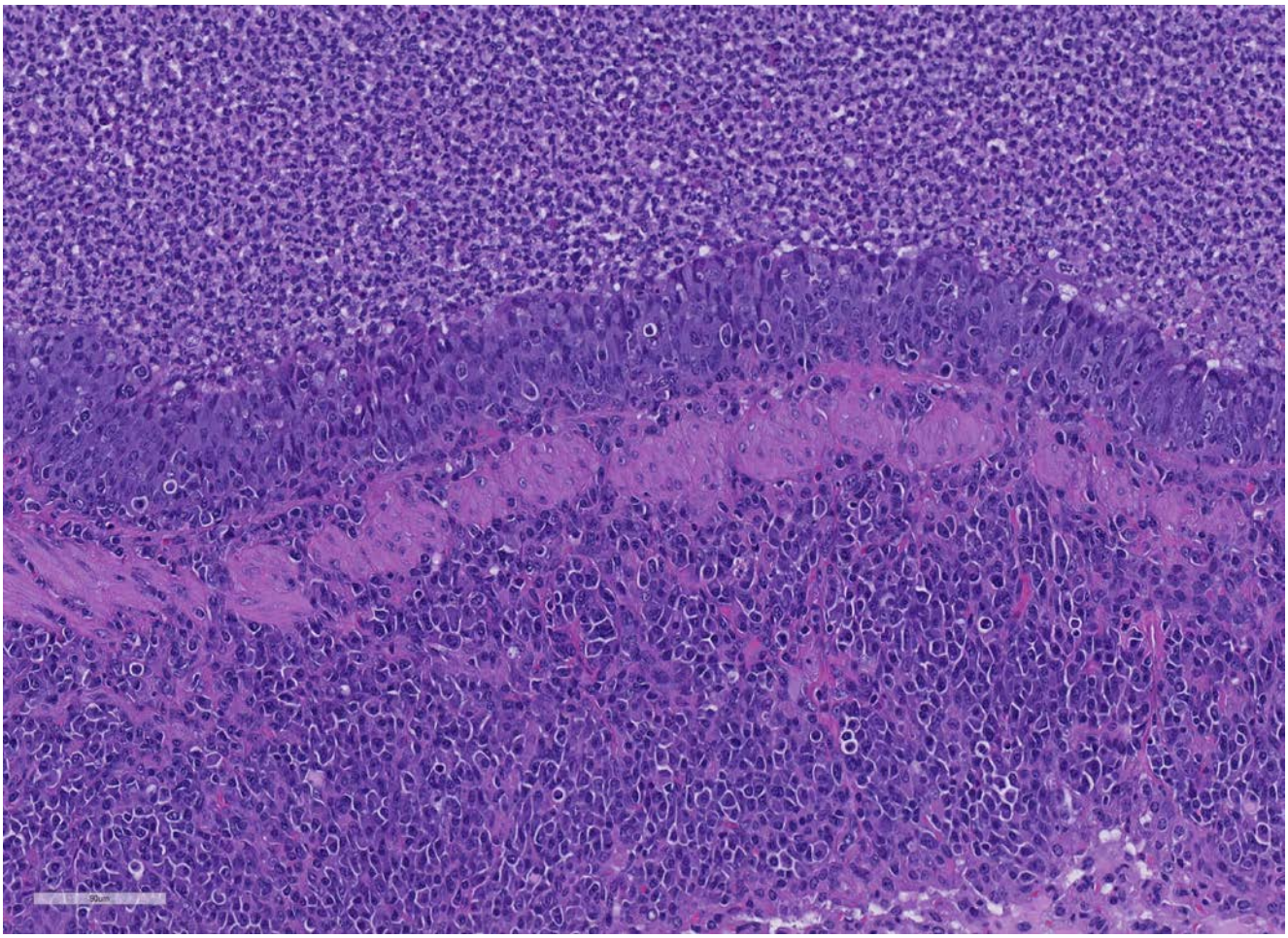
otitis. Immunodeficient mice on the other hand develop arthritis.[8]

Colonization of the respiratory tract is mediated by adhesion through special organelles of *Mycoplasma* organism to cilia, with consequent ciliostasis and retention of mucus.[7]

Grossly, mucopurulent exudate can be seen in the tympanic bulla, nasal passages and airways. When the disease is subacute or even chronic consolidation of the lung parenchyma and mottling, especially in the cranioventral regions can be appreciated.

Variably sized pearl-white to tan nodules can be observed. On cut section, they reveal a wall and a lumen partially or completely obliterated by exudate that correspond to dilated bronchial structures (bronchiectasis).

Histologically, the main and most consistent features are the luminal collection of viable and degenerated neutrophils in the nasal cavities, tympanic bullae, lower airways and the infiltration of lymphocytes and plasma cells forming cuffs around bronchi and bronchioles. Abscess formation as well as squamous metaplasia of the respiratory epithelium can be observed in chronic cases.



Lungs, mouse. The ectatic lumen (top) is filled with numerous viable and degenerate neutrophils. The hyperplastic epithelium (center) is infiltrated with neutrophils and lymphocytes, and below the wall of smooth muscle, there is accumulation of large numbers of lymphocytes and plasma cells. (HE, 242X)

The lymphoplasmacytic infiltrate is reported to be more prominent in rats than in mice.[8] The mycoplasmal membrane is decorated with high frequency variation antigens which act as superantigens, stimulating the humoral response in the infected host. Lymphoid hyperplasia around the airways is a consistent feature of the enzootic pneumonia of swine, caused by *M. hyopneumoniae.*[7]

Mycoplasma have the ability to establish infection thanks to several bacterial factors that prevent phagocytosis and killing by macrophages, which are the first line defense, and increased adherence to host's mucosal surfaces.[3,6,9] Capsular polysaccharide is an antiphagocytic factor.[4] *Mycoplasma pulmonis* produces a polysaccharide called EPS-I which has roles in cytoadherence, protection from complement, inhibition of biofilm formation.[1,2] It acts moreover as second shield defense against macrophage binding and phagocytosis. EPS-I appears to provide maximal defense against host response when present together with a long Vsa protein.[8] Vsa are a family of surface lipoproteins produced by mycoplasmas.[10,11-13] They vary in phase and size. Size variation in particular reduces binding of the bacteria by macrophages beside inhibiting biofilm formation and protecting from complement action similarly to EPS-I.

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https://www.mskcc.org/research-areas/programs-centers/comparative-medicine-pathology

JPC Diagnosis: Spleen: Splenitis, pyogranulomatous**,** diffuse, severe, with intrahistiocytic yeasts.

2. Spleen, red pulp: Granulocytic and myeloid hyperplasia, diffuse, severe.

3. Spleen, white pulp: Lymphoid hypoplasia, diffuse severe (consistent with genotype).

JPC Comment: Mycoplasma, in all their minimalized finery, embody much of what we know about what is actually necessary for the development of cellular life. In 1962, the National Aeronautic Space Administration (NASA) embarked on a continuing search for extraterrestial life, and suggesting that if found, it would be extremely simple, leading a generation of scholars to look on our own planet for what that form of life would resemble.[9] In doing so, the early investigations into mycoplasma, the simplest form of life with the ability of independent growth in artificial media, were purued.

Mycoplasa are indeed the essence of a "stripped down" life form, considered a "minimal" cell. They did not start as simple organisms at the base of the evolutionary tree which did not progress over billions of year, but evolved contrary to typical evolution, shedding large parts of its genome, as well as an independent lifestyle

for a very specialized parasitic one, becoming ever simpler and representing the absolute minimal requirements for cellular life.

Three species of mycoplasma have the smallest genome of all cellular life forms, with *M. genitalium*, a cause of urethritis in humans, having the smallest genome at 580,076 base pairs (contained in circular DNA).[5,9] In order to achieve this amazing housecleaning, mycoplasmas have sacrificed many of their genes, relegating themselves to a very particular parasitic (or in the case of some insect mycoplasmas) symbiont lifestyles.

One of the obvious results of the shedding of "excess genes" is their lack of a cell wall. Unlike most bacteria possess a cell wall, cell membrane, and cytoplasmic membrane, the evolutionary transition from their presumptive gram-positive roots, mycoplasma have jettisoned both the cell wall and cytoplasmic membrane, making themselves osmotically fragile and unable to live in the extra-organism (or extracellular environment for those species who live within other cells), but facilitating other interesting behavior such as direct fusion of their membranes with their host ro target cells to facilitate homeostatic or cytopathic endeavors).[9] (The lack of the a cell wall is also the reason why mycoplasma are resistant to many traditional antibiotics, which attack bacterial cell walls.)

Another reduction cementing their parasitic lifestyle is the deletion of all genes involved in amino acid, fatty acid and cofactor biosynthesis. Mycoplasma must receive all nutrients (and in the proper concentrations) from the host to survive, a feautre that prevented their growth in the laboratory for many years. Many continue to be fastidious in their requirements, often failing to grow as a result of mild "overdosing" of required amino acids or other nutrients in media.[9] (They are however, excellent parasites of cell cultures, with estimates of up to as many of 80% of cell cultures globally containing living mycoplasmas as "part of doing business".)[9]

Additional gene deletions have affected energy metabolism, leaving mycoplasmas to depend on glycolysis as their main, however ineffective means of energy production. They also are lacking in genes coding for elements of the Kreb's cycle or any cytochromes, damning these processes as "nice to have" but not essential for cellular life.[9]

One other way that mycoplasmas have adopted a stripped down life cycle is their lack of "redundant" genes. For most cellular functions in traditional bacteria, such as E.coli, each function as built-in redundancy, with anywhere from 2-6 separate genes for the same function. Mycoplasmas in generally possess a single gene for each function, which when knocked out as part of genomic investigation, most often results in death of the cell, or rarely, revision to non-pathogenicity.[9]

With all of this loss of genetic material, it is interesting that *M. genitalium*, the cellular organism with the smallest genome in the world, yet possesses twice the number of genes that investigations in the "minimal

cell set" described by molecular biologists as the requirements for life and reproduction.[5] It would appear that *Mycoplasma* as a genus still has some housecleaning to do.

One of the most interesting morphologic changes in this particular slide is the marked hyperplasia of bronchiolar epithelium which appears to extend into adjacent alveoli by lepidic growth. While the cause of this change is not evident, the moderator and other renowned mouse pathologists with whom she consulted on this case suggested the possibility of a concurrent virus which may may not have been tested for, such as Sendai or pneumonia virus of mice.

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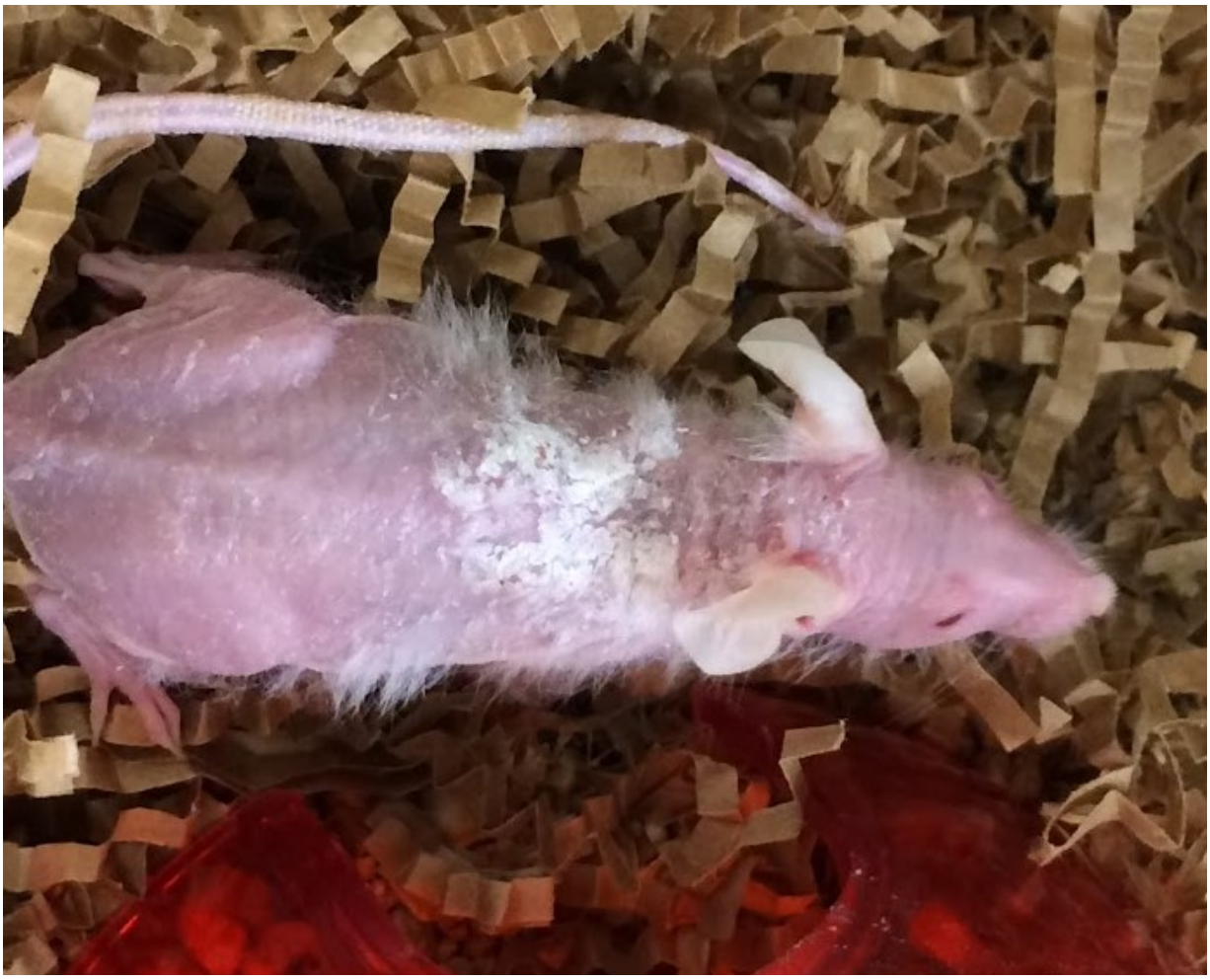
CASE IV: 17N076 (JPC 4103914).

Signalment: ~6 months; Female; NOD.*Cg-Prkdc*^{scid} *IL2rg*^{tm1Wjl}/SzJ (aka NOD-*scid*-gamma, NOD-*scid* IL-2Rγ^{null}, NSG™ mouse); *Mus musculus*

History: A NOD-*scid*-gamma (NSG™) mouse was presented for progressive diffuse hair loss and scaling dermatitis. (NSG™



Presentation, NSG IL-2 Rg null mouse. The mouse has complete alopecia affecting the entire hair coat, with scaling dermatitis predominantly on the interscapular dorsum (Photo courtesy of: Unit for Laboratory Animal Medicine, In Vivo Animal Core, University of Michigan, 2800 Plymouth Road, B36-G178, Ann Arbor, MI 48109 http://animalcare.umich.edu/business-services/vivo-animal-core)

mice are typically fully haired). Four months prior to presentation, the mouse had undergone chemical depletion of the native hematopoietic compartment and adoptive transfer of human $CD3^+$-depleted bone marrow mononuclear cells, followed 2 weeks later by human $CD3^+$ T cell transfer. The experimental intent was to create a humanized hematopoietic system, which could then be used to assess effects of experimental agents on the hematopoietic system.

Gross Pathology: The mouse had complete to partial alopecia affecting the entire hair coat, with scaling dermatitis predominantly on the interscapular dorsum

Laboratory results:

- PCR was performed on skin using primers specific for *Corynebacterum bovis*: results were negative
- Aerobic culture of skin grew few - *Staphylococcus aureus* and rare *Enterococcus faecium*

Microscopic Description: Skin: In sections of haired skin, there is multifocal vacuolar change within the basilar epidermal and adnexal epithelium, evidenced by shrinking and separation of adjacent keratinocytes with occasional intracytoplasmic vacuolation and occasional dyskeratosis. Shrunken cells with pyknotic nuclei, consistent with apoptotic keratinocytes, are multifocally present in the same areas. Additionally, there is a mild to moderate interface and adnexal dermatitis comprised of lymphocytes and macrophages adjacent to and infiltrating the basal epithelium, outer root sheath of follicular epithelium, and sebaceous gland epithelium. Multifocal lymphocytic and macrophagic dermal infiltration and mild dermal fibrosis are also present. The overlying epidermis has moderate diffuse orthokeratotic hyperkeratosis and the stratum granulosum is prominent (hypergranulosis).

Other organs evaluated (not shown) included the lungs, liver, kidneys, uterus, spleen, mesenteric lymph nodes, pancreas, gastrointestinal tract, and bone marrow. The lungs contained diffuse peribronchial and peribronchiolar lymphocytic infiltration, with occasional intraepithelial infiltrates. The liver and kidneys showed infrequent, perivascular and peribiliary (in liver) mononuclear to lymphocytic infiltration. The bone marrow, spleen, and lymph nodes were highly cellular with appropriate distribution of erythroid and myeloid precursors in marrow and red pulp and with appropriate lymphoid cellularity and organization in the spleen and lymph nodes.

Contributor's Morphologic Diagnosis:

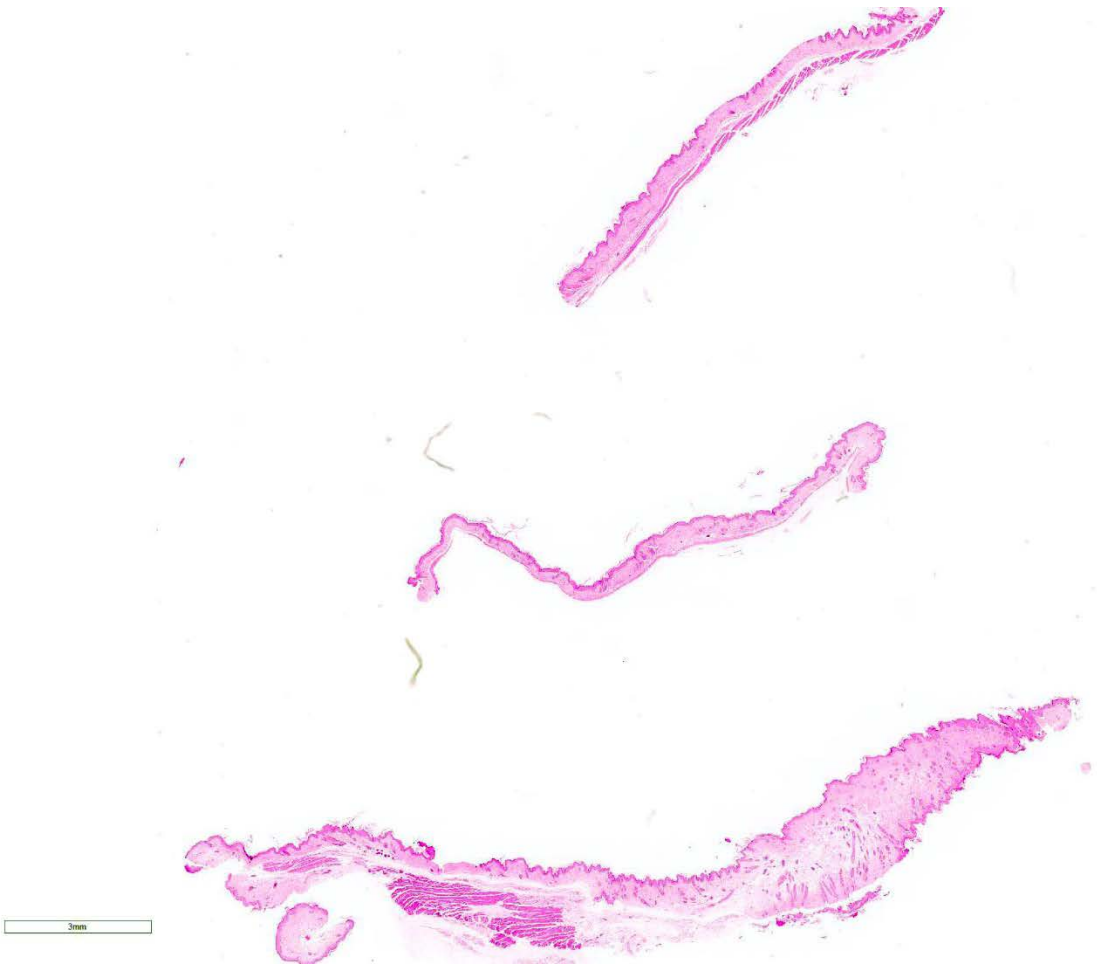
Skin: Dermatitis, interface and adnexal, lymphohistiocytic, with basilar vacuolar change, orthokeratotic hyperkeratosis, and dermal fibrosis, chronic, multifocal, moderate

Contributor's Comment: The gross and histologic findings in this case were consistent with graft-vs-host disease secondary to a protocol intended to generate immunologically "humanized" mice.

NSG™ mice[2,7] have three immune-related defects, consisting of:

1) The genetic background "NOD/ShiLtJ", which is a polygenic defect of the innate immune system resulting in impaired phagocytosis and defective antigen presentation by macrophages and dendritic cells
2) Severe combined immunodeficiency ("scid") mutation arising from loss of function of the gene *Prkdc*. *Prkdc* encodes the catalytic subunit of the protein complex that controls ligation of V(D)J DNA fragments during T cell receptor or immunoglobulin gene recombination in lymphocytes. Its absence results in failure to generate mature, functional B or T lymphocytes.
3) A targeted null mutation of the IL2 receptor γ chain, which blocks receptor recognition of cytokines IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21. This further impacts murine hematopoietic development and results in a lack of mature, functional NK cells (development requires IL-15).

Because of these broad immunologic defects, NSG™ mice are often the recipient strain of choice for experimental engraftment, including the transfer of human



Haired skin, NSG IL-2 Rg null mouse. Three sections of skin are presented for examination. (HE, 6X)

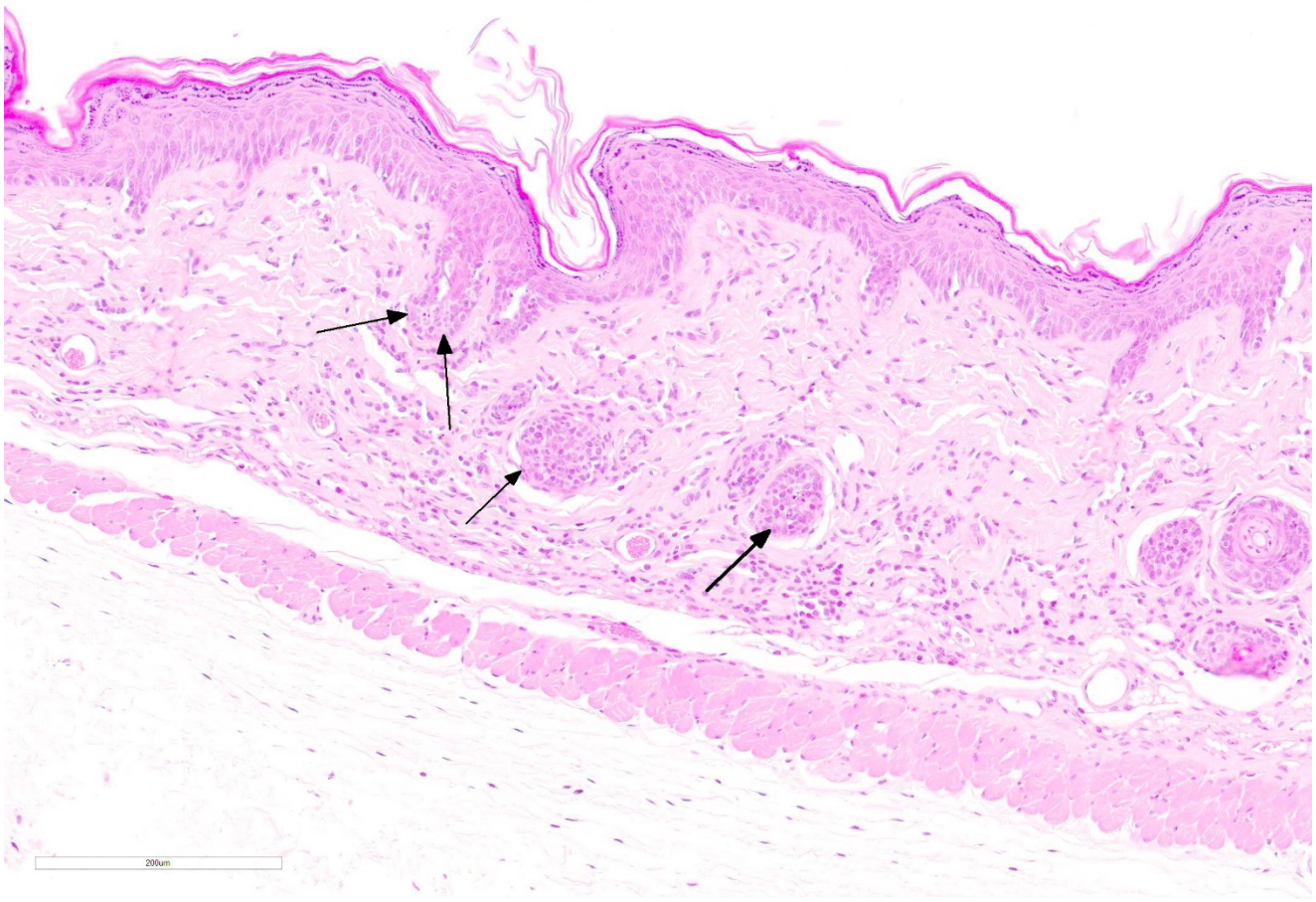
hematopoietic cells to generate mice with a "humanized" immune system.[2,7] These humanized mouse protocols involve irradiation or chemical depletion of the native murine hematopoietic compartment and transfer of either human fetal bone marrow, liver, and thymus (BLT) or human bone marrow or peripheral blood-derived $CD34^+$ stem cells. Engrafted cells migrate to bone marrow and differentiate to all lineages of the mature immune system.

Humanized mouse protocols carry the potential for development of graft-vs-host disease (GVHD), in which human donor T cells are primed against murine antigens presented by graft-derived human cells, generating an immune response against murine host tissues. To avoid this, grafts are typically depleted of mature CD3+ cells to allow immature T cells, which will ostensibly regard host tissues as "self", to develop from the graft. Nevertheless, GVHD has been reported in humanized NSG™, most commonly in mice receiving BLT, but occasionally in mice receiving $CD34^+$-selected, $CD3^+$-depleted stem cells grafts.[2-5] Engraftment of non-$CD3^+$-depleted, peripheral blood mononuclear cells (PBMCs) has also been used to purposefully induce GVHD for study of pathogenesis or interventional strategies.[1,3]

In this mouse, the most severely affected organs were the skin and the lung. Organs affected by GVHD in NSG™ mice resemble those targeted in human GVHD, with skin, lung, liver, gingiva, and intestinal tract variably affected, depending on the type of graft, the individual mouse, or the stage of disease.[3,6] Organ specificity may relate to T cell homing receptor expression.[1] GVHD can occur acutely or have a more insidious, chronic course. Chronic GVHD may have a more complicated pathogenesis involving autoantibody production and impaired development of central or peripheral tolerance in graft-derived T cells. In both acute and chronic GVHD, MHC I-mediated presentation of murine antigen by graft-derived cells plays a key role, as GVHD preferentially occurs in mice receiving human grafts with specific HLA class I antigen haplotypes, particularly those associated with autoimmune disease in humans.[5] GVHD in this mouse clinically resembled chronic GVHD as it occurred 4 months after grafting- this is much longer than the typical presentation of acute GVHD in NSG mice (3-4 weeks post-engraftment).[9]

The key feature of cutaneous GVHD is interface dermatitis with basilar keratinocyte apoptosis.[5] This ranges from a mild interface dermatitis with vacuolar change to a lichenoid (band-like) infiltration. In chronic GVHD, orthokeratotic hyperkeratosis and dermal fibrosis (dermal sclerosis) become more prominent and inflammation may be more severe, although there is considerable variation and features of acute and chronic GVHD may be present in the same individual.[5]

The key feature of chronic pulmonary GVHD in humans is the appearance of bronchiolitis obliterans, believed to result from chronic inflammation and fibrotic remodeling of small airways. In this mouse case, bronchiolitis obliterans was not apparent and changes were confined to a



Haired skin, NSG IL-2 Rg null mouse. There is mild lymphohistiocytic interface dermatitis of the epidermis, follicles, and adnexa; and intercellular edema of the the basal epithelium. (HE 400X) ((Photo courtesy of: Unit for Laboratory Animal Medicine, In Vivo Animal Core, University of Michigan, 2800 Plymouth Road, B36-G178, Ann Arbor, MI 48109 http://animalcare.umich.edu/business-services/vivo-animal-core)

pronounced lymphohistiocytic bronchiolitis. It may be that fibrotic changes of the airway would develop with time. A recent survey of human patients with clinically suspected GVHD-associated bronchiolitis obliterans showed that 9 of 33 biopsies showed only lymphocytic bronchiolitis without fibrosis – this may represent an earlier stage of disease progression.[5]

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JPC Diagnosis: Haired skin: Dermatitis, lymphohistiocytic, diffuse, mild to moderate, with epidermal and follicular basal cell apoptosis, intra- and extracellular edema, epidermal hyperplasia, hypergranulosis, and orthokeratotic hyperkeratosis.

JPC Comment: For a wide range of hematologic malignancies, a cure is only availble through the use of allogeneic hematologic stem cell transplants (allo-HSCT).[4,7,8] Over a million of these procedures have been completed within the last decade[4], in which a combination of radation and chemotherapy is used to eliminate neoplastic or genetically abnormal cells of the hematopoetic compartment which are then replaced with hematopoetic stem cells from an allogeneic donor possessing differences in human leucocyte antigens (HLA) or major (MHA) and minor histocompatibility antigens (miH).[4] In the most simple terms, the syndrome of graft-versus host disease results from when cells from an immunocompetent donor recognize and attack the tissues of the immunocompromised recipient (who is incapable of mounting a response against the donor's cells). GHVD is a major cause of morbidity in allo-HSCT recipients, affecting up to 40-60%, and also accounting for 15% of mortality.[7]

In humans, cutaneous GVHD is considered the earliest and most common manifestation and may wraant a poor progosis.[4,7] A macular rash affecting a number of body parts, including the palms and soles, is its hallmark. This condition, in addition to vomiting and jaundice resulting from small bile duct damage and cholestasis, forms the basis for early diagnosis of acute GVHD.[8]

GVHD is divided into classic acute GVHD in which symptoms appear within 100 days (often 2-3 weeks following engraftment) and are consistent with the triad described above, and late onset acute GVHD which also shares these symptoms but appears after 100 days. Chronic GVHD represents 50% of all cases and 25% of mortalities occurs after the 100 day period.

In humans, the rash seen in aGVHD are usually centered on hair follicles, a useful early sign for diagnosis.[4,7] Histologically, the lesion in humans is very similar to that seen in the mouse model (and in this case), with vacuolar degeneration of the basal layer, dyskeratotic keratinocytes, and a mild lymphocytic infitrate at the dermo-epidermal junction that may infiltrate the epidermis beginning at the rete ridges and hair follicles.[7] The population of lymphocytes is predominantly $CD4^+$ and $CD8^{+,}$ and their presence in the epidermis suggests that they are donor lymphocytes attacking keratinocytes expressing different MHA antigens.[8]

Chronic forms of cutaneous GVHD (cGVDH) appear to be more common overall, and are reported in between 60 and 80% of patients. The most commonly recognized forms include lichenoid GVHD and sclerodermatous cGVHD. Lichenoid cGVHD exhibits most of the histologic signs descrbed about, with a macular hyperkeratotic rash. Sclerodermatous cGVHD may result in fibrosis at any level of the skin and ultimately may be disfiguring.[7]

One positive aspect of GVHD is associated with an effect known as graft-versus-leukemia (GVL). It well-documented that these patients have lower relapse rates of hematologic malignancies, and is a useful benefit in paitents with reduced intesity regimens to clear the body of neoplastic hematologic cells.[4]

Many participants had *Corynebacterium bovis* on ther list of differentials, but the moderator pointed out the lack of follicular hyperkeratosis, the mininal inflammation, and the proliferation of the stratum spongiosum, rather than the stratum granulosum, as in this case.

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