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Case Report

Presumptive Spontaneous Lysosomal Storage-Like Disease in a Crl:CD1(ICR) Mouse

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A clinically unremarkable 4.5-mo-old female Crl:CD1(ICR) VAF/Elite mouse was euthanized for scheduled sentinel processing. Gross necropsy findings included significant hepatosplenomegaly and visceral lymphadenomegaly, resulting in a preliminary gross diagnosis of lymphoma. Histology revealed florid accumulations of large, 'foamy' macrophages present in the bone marrow, small intestines, and viscera including liver, spleen, lymph nodes, thymus, uterus, and ovaries. The cytoplasm of these cells was abundant, stained pale blue with Wright–Giemsa and was periodic acid–Schiff positive. Given these characteristic gross and histologic findings, a spontaneous lysosomal storage-like disease was diagnosed in this mouse. Cholesterol ester storage disease is likely, because of the visceral involvement with sparing of the CNS, but could not be diagnosed definitively. To our knowledge, this report is the first to describe a case of spontaneous lysosomal storage disease in an outbred mouse of the CD1(ICR) background.

Abbreviations: LAL, lysosomal acid lipase; LSD, lysosomal storage disease

The term 'lysosomal storage disease' (LSD) refers to a broad category of rare metabolic disorders that result from defects in the enzymes used to break down various macromolecules in cells. The majority of these diseases are hereditary, but acquired forms can develop after plant intoxication (through inhibition of α-mannosidase II) or the administration of pharmaceuticals (for example, amphophilic cationic drugs).[1] Although most LSD are caused by the loss of function of a specific lysosomal acid hydrolase, a defect in any of the participating enzymes in the stepwise process can result in the upstream accumulation of partially degraded or nondegraded substrate molecules.[4,9,12] Because of the various enzymes that participate in the process, LSD are classified according to the macromolecule that accumulates within the cell, which directly correlates to the enzyme deficiency within the subject.[8,9,11] The accumulation of these molecules often interferes with cellular processes and transport systems, and may result in cell death.[12]

The age of onset and severity of LSD are dependent on the specific genetic defect in the affected subject. Some mutations result in complete loss of enzyme function, whereas others only dampen enzyme function (that is, hypomorphic phenotype). Complete loss of function generally results in a rapid onset of severe disease, whereas patients who retain residual enzyme function sustain normal cellular functions over prolonged periods, resulting in milder clinical signs.[8,12] Because most LSD are inherited in an autosomal recessive pattern, only those subjects that are homozygous for the mutant allele manifest the disease. However, most LSD result in a gene-dose–dependent phenotype, such that enzyme function in heterozygotes is approximately 50% of normal levels.[8,12]

More than 40 human inherited lysosomal storage diseases have been described, many of which have also been documented to occur spontaneously in animals.[4] Spontaneous cases of lysosomal storage diseases have been noted most frequently in specific breeds of canines and felines, but other large animal models, including bovine, ovine, porcine, and caprine, have proven exceptionally helpful in the study of these diseases.[4,8,12] However, as with most animal models, working with spontaneous cases of lysosomal storage diseases has its limitations, particularly their infrequent occurrence and the presence of variable modifier genes in outbred animals. Consequently, thorough characterization of inbred strains and advancements in genetically engineered mouse models have enabled researchers and clinicians to elucidate genetic and biochemical mechanisms associated with these diseases in a more predictable manner.[8] This understanding has led to the development of targeted therapies and screening techniques that are used not only in veterinary medicine but that have translated into human clinical medicine as well.[4,8] From a research standpoint, large animal models are extremely beneficial, particularly with regard to testing gene therapies, because they tend to portray the human condition more accurately in terms of size, genetic heterogeneity, and longevity, compared with genetically engineered inbred mice.[4]

Here we describe a 4.5-mo-old outbred Swiss mouse that presented for scheduled sentinel evaluation. The hepatosplenomegaly and lymphadenomegaly present on gross examination were typical of lymphoma, a common cause of morbidity and mortality in inbred and outbred mice.[12, 13] Lymphoma has been reported to occur in CD1 female mice as young as 3.5 mo and has

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occasionally been diagnosed in sentinel mice in our institution.[12,15,16] Surprisingly, the histopathology of the current case revealed lesions consistent with lysosomal storage disease. This case acts as a reminder to consistently evaluate the overall health of a mouse with gross abnormalities and its suitability as a sentinel animal, given that unexpected pathology has the potential to influence interpretation of testing results (particularly serology).

Case Report

A 6-wk-old female CD1-Elite (Crl:CD1[ICR] VAF/Elite, strain code 482) mouse arrived from the vendor (Charles River Laboratories, Wilmington, DE) and was housed in an IVC with a female conspecific within a barrier facility at the Penn State College of Medicine, an AAALAC-accredited institution. The mouse was on an IACUC-approved protocol, and husbandry was performed in accordance with the *Guide for the Care and Use of Laboratory Animals*[7]. Dirty-bedding sentinel sampling began after 1 wk of acclimation and continued for 3 mo. For this purpose, samples of dirty bedding from 10 to 14 cages were placed in the sentinel cage every 2 wk for exposure. During this time, the mouse had free access to standard rodent chow (Teklad 2919, Envigo, Dublin, VA) and water. According to previous serology testing (Opti-Spot Mouse Basic Panel, IDEXX BioResearch, Westbrook, ME), mice in this room were free from ectromelia virus, rotavirus, lymphocytic choriomeningitis virus, *Mycoplasma pulmonis*, mouse hepatitis virus, mouse norovirus, mouse parvovirus, minute virus of mice, pneumonia virus of mice, reovirus 3, Theiler murine encephalomyelitis virus, and Sendai virus. The room housing this mouse had tested positive for enzootic diarrhea of infant mice virus approximately 1 y previously; the virus was successfully eradicated after a breeding moratorium and test-and-cull procedures.

The mouse we describe was euthanized by carbon dioxide asphyxiation followed by cervical dislocation after 12 wk of exposure to dirty bedding for sentinel sample collection, which includes cardiocentesis for antibody testing (Opti-Spot, IDEXX BioResearch, Columbia, MO) but not whole-blood collection for serum banking. Upon opening of the abdomen for collection of cecal contents, fresh feces, and mesenteric lymph node, the liver and spleen were notably enlarged, and the veterinary staff was contacted. A preliminary diagnosis of lymphoma was made, and the mouse was submitted for complete pathologic evaluation. The conspecific female mouse from this cage was grossly normal at time of sentinel sample collection.

Gross Pathology

On gross necropsy, the liver (weight, 6.16 g) and spleen (0.52 g) were diffusely enlarged with rounded edges (Figure 1 A). The liver was diffusely pale and sank in formalin. A single, soft, white amorphous mass (dimensions, $4 \times 8 \times 2$ mm) was present in the mesentery, which was later identified as a pancreaticoduodenal lymph node (Figure 1 B). The walls of the duodenum were moderately thickened and diffusely yellow in color. In addition, tracheobronchial lymph nodes were enlarged. Wright–Giemsa-stained touch impressions of the liver revealed numerous large foamy macrophages, with fewer lymphocytes and neutrophils. Hepatocytes present in the sample were normal.

The pelt was submitted for ectoparasite examination and was not available for histology. All remaining organs and tissues were collected and fixed in 10% neutral buffered formalin, and the head and hindlimb were skinned and placed in a fixative decalcifier (Formical 4, American Master Tech, Lodi, CA). After fixation, tissues were routinely processed, paraffin-embedded, and stained with hematoxylin and eosin. Tissues examined included lungs, liver, spleen, kidneys, adrenal, thymus, larynx, trachea, esophagus, thyroids, heart, salivary glands (sublingual, submandibular, and parotid), stomach, duodenum, pancreas, small intestine (jejunum), colon, uterus, oviducts, ovaries (with bursa), urinary bladder, mammary fat pad, and pancreaticoduodenal, submandibular, and tracheobronchial lymph nodes. In addition, 6 coronal sections of the decalcified head and 2 sagittal sections of the decalcified hindlimb were reviewed. After preliminary examination of the slides, additional slides containing liver, spleen, small intestine, and the mesenteric mass were stained with Wright–Giemsa, periodic acid–Schiff, and Ziehl–Neelsen acid-fast stains. In addition, a 3-µm section of kidney was cut and stained with periodic acid–Schiff. All slides were reviewed by a board-certified veterinary pathologist (TKC). All images were obtained by using a model BX51 microscope, DP71 digital camera, and cellSens Standard version 1.12 imaging software (Olympus America, Center Valley, PA).

Histopathologic Findings

The mesenteric mass was determined to be an enlarged pancreaticoduodenal lymph node, which was markedly expanded by the infiltration and accumulation of myriad large macrophages with abundant foamy cytoplasm within the medullary and subcapsular sinuses, with compression of normal tissues (Figure 2 A and B). The liver sinusoids were diffusely and markedly expanded by coalescing small nodular accumulations of similar large, foamy macrophages (Figure 2 C). Intervening hepatocytes were normal. Similarly, the splenic red pulp was markedly expanded by coalescing small nodular accumulations of foamy macrophages. These cells similarly expanded the medullary and subcapsular sinuses of tracheobronchial and submandibular lymph nodes. There were occasional small nodules of foamy macrophages present in the cortex and medulla of the thymus. Larger accumulations of these cells were present diffusely within the lamina propria of the duodenum and some sections of jejunum, expanding the villi and separating the crypts, and expanding the submucosa (Figure 2 D). Foamy macrophages also were present within Peyer's patches, as was the female reproductive tract, with accumulations of these cells in the deep endometrium and myometrium and within the stroma of both ovaries. Histologic evaluation of the decalcified head and hindlimb revealed moderate accumulations of foamy macrophages in the bone marrow. The brain, retina, peripheral nerves, skeletal muscle, bone, cartilage, and haired skin (foot) were all unaffected. Atherosclerosis, a common feature of several human lysosomal storage diseases, was not present.

Special histochemical staining of these cells revealed abundant pale-blue cytoplasm in the macrophages (so-called 'sea-blue histiocytes') when stained by Wright–Giemsa (Figure 2 E). The material present in the cytoplasm of the macrophages was positive for periodic acid–Schiff staining (Figure 2 F). Acid-fast staining (Ziehl–Neelsen) was negative for all tissues tested.

Discussion

In general, LSD are characterized by the onset of progressive neurologic impairment beginning at a young age.[4,8,11] This

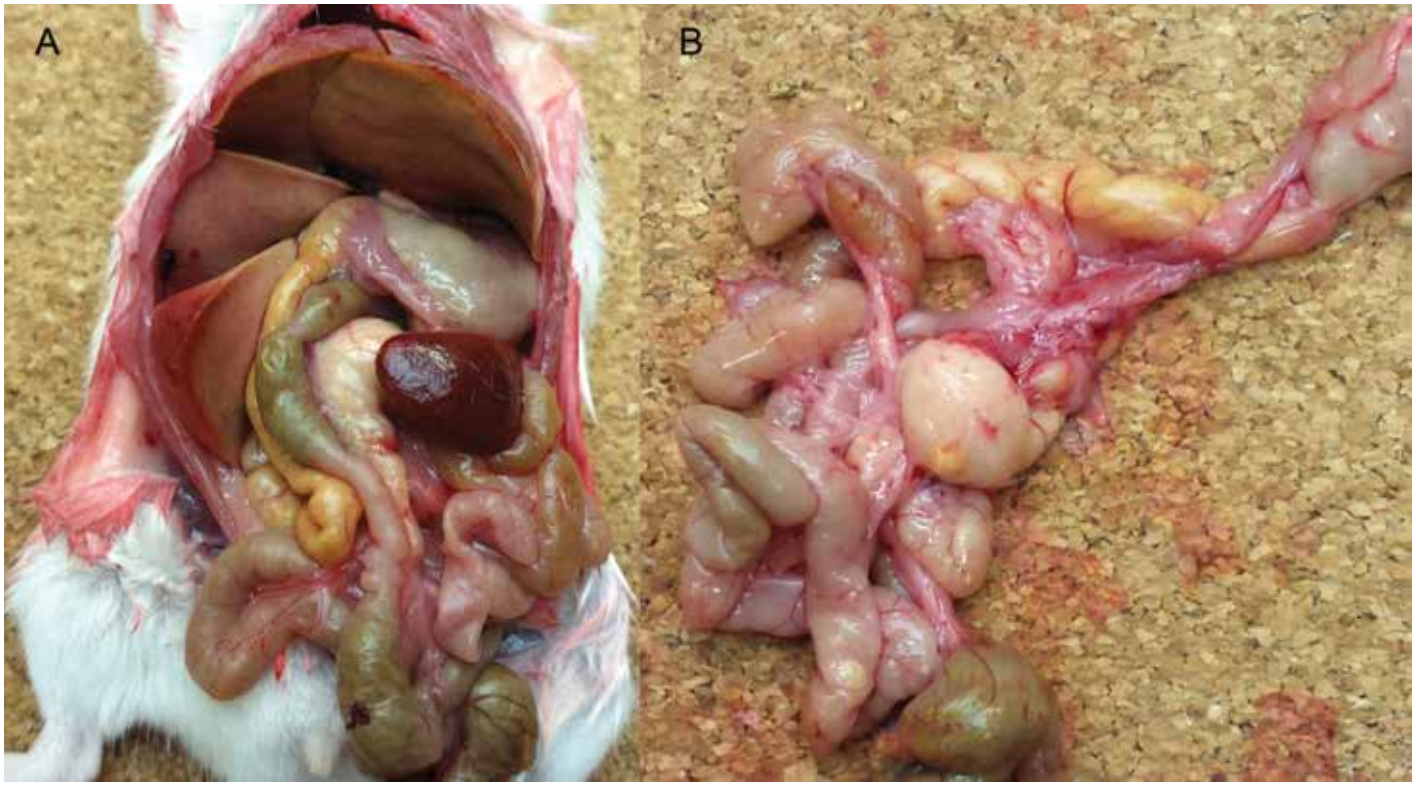

Figure 1. (A) Gross hepatosplenomegaly and pallor of the liver. The soft mesenteric mass (later identified as the pancreaticoduodenal lymph node) can be seen to the left of the spleen, and the yellowing of the duodenum and proximal jejunum and proximal small intestine is also evident. (B) Explanted gastrointestinal tract exposing the single mesenteric mass (center of the photo) and yellowing of the walls of the duodenum.

symptomatology results because many lipids and glycolipids are unique to the neurons and myelinating cells, and cellular dysfunction in this location can be devastating. When these cells die, they release the accumulated material, and the material is phagocytosed by macrophages with the same dysfunction.[12] The resulting cellular expansion, necrosis, and inflammation occurring in such a vital location results in the neurologic and often fatal impairment seen with most LSD. Not all LSD affect the nervous system; a few have multisystem effects that spare the neural tissue, including cholesterol ester storage disease, sphingomyelinosis (Niemann–Pick disease type B, specifically), and some glycogen storage diseases. Glycogen storage diseases that spare the neural tissues are characterized by accumulations of glycogen products within the hepatocytes or skeletal and cardiomyocytes.[9] Because the brain was spared in the mouse presented here and given the lack of skeletal or cardiac muscle cell involvement, the differential diagnoses were narrowed down to cholesterol ester storage disease and Niemann–Pick disease type B. Both of these conditions are autosomal recessive in nature.[5,14,15]

Cholesterol ester storage disease is a lysosomal storage disorder caused by mutation of the *Lipa* gene.[5,14] This mutation results in a deficiency of lysosomal acid lipase (LAL), the enzyme responsible for the hydrolysis of cholesterol esters and triglycerides within lysosomes.[14] LAL dysfunction results in an accumulation of these substrates within macrophages of the liver and spleen (predominately).[3,8] In humans, LAL defects result in 2 distinct phenotypes: Wolman disease and cholesterol ester storage disease. Similar to other LSD, the expressed phenotype is dependent on the complete or partial loss of the functional enzyme. Complete loss of LAL results in Wolman disease, which is characterized by in an early onset of severe disease, often resulting in death before 1 y of age. A similar disease described in fox terriers likewise results in death at younger than 1 y.[8]

Cholesterol ester storage disease is a milder, later-onset form of LAL deficiency than is Wolman disease and may present during childhood or later in life. This disease is suspected in persons with hepatomegaly, elevated transaminases, and high total serum concentrations of cholesterol and triglycerides; however, cholesterol ester storage disease is thought to be underdiagnosed given the overlap of its clinical and biochemical signs with those of autosomal recessive hypercholesterolemia and nonalcoholic fatty liver disease.[5,14]

A mouse model of LAL deficiency has been created (*Lipa*^{tm1Ggb}) and bears striking phenotypic resemblance to the mouse in the current case.[3,14] *Lipa*^{tm1Ggb} mice appear normal at birth, but over time the storage of triglycerides and cholesterol esters within the liver, adrenal gland, and small intestines typically results in mortality at 7 to 9 mo of age. Perhaps the most interesting similarity between the outbred mouse we describe here and the *Lipa*^{tm1Ggb} mice is the enlargement of a single mesenteric lymph node, which was due to stored lipids within macrophages rather than to lymphocyte proliferation. As reported by the group that developed the *Lipa*^{tm1Ggb} mice, this feature arose only in mice that were older than 2 mo.[3] An additional similarity between the presented and *Lipa*^{tm1Ggb} mice is the involvement of the small intestine, including yellowing of the duodenum. However, unlike those in *Lipat*^{tm1Ggb} mice, the amounts of adipose tissue were grossly normal in the current case.

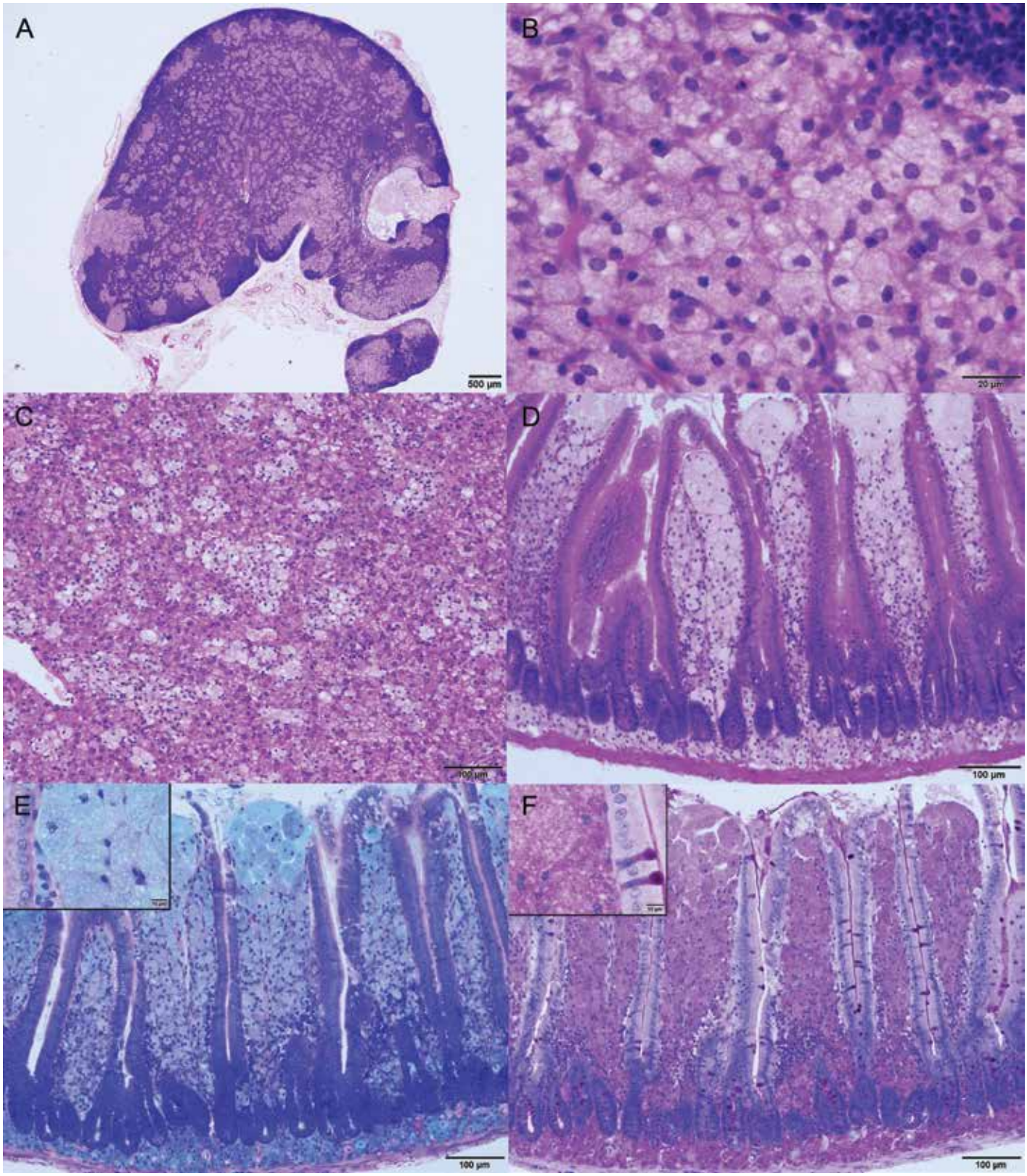

Figure 2. (A) The mesenteric mass is identified as a lymph node with distorted and compressed normal architecture. Hematoxylin and eosin stain; bar, 500 µm. (B) The mesenteric pancreaticoduodenal lymph node is markedly expanded by large accumulations of myriad foamy macrophages in the subcapsular and medullary sinuses. Hematoxylin and eosin stain; bar, 20 µm. (C) The hepatic sinusoids are diffusely and markedly expanded by coalescing small nodular accumulations of large foamy Kupffer cells. There is a mild amount of micro- and macrovesicular lipid accumulation within the

Despite the remarkable similarities between this CD1Elite mouse and the *Lipa*^{tm1Ggb} mice, LAL deficiency could not be definitively diagnosed in the current case. Due to an overlap in the histologic similarities, an alternative diagnosis for this mouse is sphingomyelinosis (Niemann–Pick type B, specifically). Sphingomyelinosis results from a mutation of the *Smpd1* gene and refers to related disorders characterized by a deficiency of sphingomyelinase.[9,15] Sphingomyelinase is responsible for the cleavage of sphingomyelin into phosphocholine and ceramide within lysosomes. When the enzyme is nonfunctional, sphingomyelin (a major component of cell membranes) accumulates within the cells, resulting in large, lipid-laden cells within the liver, spleen, lymph nodes, adrenal cortex, lung airways, and bone marrow, which are characteristic pathologies of Niemann–Pick types A and B.[15] Niemann–Pick disease has been described in Siamese, domestic shorthair, and Balinese cats as well as poodle and boxer dogs.[11] Most of these cases are most similar to Niemann–Pick disease type A.

Niemann–Pick type A is considered the more severe form of the disease and is characterized by hepatosplenomegaly and profound neurologic involvement beginning in infancy.[15] Children diagnosed with type A disease often die within the first 4 y of life. In contrast, the age of onset and rate of disease progression of Niemann–Pick type B disease are quite variable. In addition, patients with the type B form usually survive to adulthood with organomegaly and pulmonary changes but no CNS involvement.[8,9,15] Similar to those with cholesterol ester storage disease, patients with type B often have elevated levels of serum triglycerides and LDL-cholesterol.[15]

Cases of spontaneous Niemann–Pick type B disease have not been characterized in veterinary species, although genetically engineered mouse models (*Smpd1*^{tm1Esc}) are available. Despite being more similar to patients with type A disease, *Smpd1*^{tm1Esc} mice are frequently used for the study of both diseases. Homozygous mice exhibit progressive lipid storage within the mononuclear phagocyte system and brain. Affected mice die within 6 to 8 mo, and death is presumed to be due to the neurologic effects.[6] A transgenic mouse model of type B disease was developed by introducing a partially functional mouse *Smpd1* gene into the complete knockout background; the resulting mice never developed a neurologic phenotype and lived a normal lifespan.[10]

In our current case, clinical history, histologic features, and special stains helped to rule out several differential diagnoses. For example, foamy macrophages can result from the accumulation of lamellar bodies (also called lysosomal inclusion bodies). These structures are a hallmark of drug-induced phospholipidosis, which can occur after the administration of several classes of drugs, including antibiotics, antidepressants, antipsychotics, and antimalarial and antiarrhythmic drugs.[2] Given this mouse's well-known history, including lack of drug administration, lamellar bodies could be ruled out. In addition, the crisp, sharp borders of the vacuoles within the macrophages were inconsistent with the most common of the lysosomal storage diseases in humans, Gaucher disease type 1.[8] The type 1 form of disease results in the accumulation of glucocerebrosides within macrophages, which acquire a classic fibrillary type of cytoplasm, resembling crumpled tissue paper.[1]

Cytoplasmic accumulations within macrophages can be indicative of atypical (nontuberculous) *Mycobacterium* infection. However our use of Ziehl–Neelsen acid-fast stain excluded that possibility. Periodic acid–Schiff reactions stain carbohydrates, thus facilitating the identification of the materials accumulating intracellularly. Finally, Wright–Giemsa stain displayed the 'sea-blue histiocytosis' classic of abnormal lysosomal storage processes, particularly LAL deficiency. Definitive diagnosis would require additional testing modalities that were infeasible in the current case, such as specifically evaluating LIPA and SMPD1 function in circulating leukocytes, demonstrating heritability through test breedings, or sequencing the relevant genes. Unfortunately serum or blood was not saved in this case, because a preliminary diagnosis of lymphoma was made and serum chemistry was expected to be uneventful. No unfixed tissues were saved for lipid staining of frozen sections or sequencing, and complete sequencing of both candidate genes to identify a novel mutation was impractical.

In conclusion, rather than the expected and common diagnosis of lymphoma, this mouse was determined to have a spontaneous lysosomal storage-like disease. The results we report here are noteworthy because this case represents the first known incidence of a spontaneous lysosomal storage-like disease in an outbred mouse of the CD1(ICR) background. Because of the autosomal recessive inheritance pattern of these deficiencies, we infer that the predecessors and progeny of this mouse (and related litters) include carriers of the causative mutation. In addition, the negative serology results from this mouse highlight the importance of cautious interpretation, given the potential for accumulated materials within the macrophages to interfere with normal antigen presentation to lymphocytes. Although the validity of the serology for this mouse was not specifically explored, this case serves as an important reminder to evaluate the reported results of sentinel testing in light of the overall health status of the mouse used.

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cytoplasm of the hepatocytes. Hematoxylin and eosin stain; bar 100 µM. (D) Large accumulations of foamy macrophages are present in the duodenal lamina propria, which expand the villi, separate the crypts, and expand the submucosa. Hematoxylin and eosin; bar, 100 µm. (E) The macrophages in the duodenum contain abundant 'sea-blue' cytoplasm. Wright–Giemsa stain; bar, 100 µm (inset, 10 µm). (F) The material present in the cytoplasm of the duodenal macrophages is positive by periodic acid–Schiff staining; bar, 100 µm (inset, 10 µm).

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