Thomas M. Donnelly, DVM, Column Editor

What's your diagnosis?

Cachexia in a B6;129S2-*Tnfsf5*^{tm1Imx} Mouse

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Animal technicians reported a 16-week-old female B6;129S2-*Tnfsf5*^{tm1Imx} mouse for a primary complaint of cachexia. Clinical signs included lethargy, trembling, low body temperature, anorexia, and weight loss. The animal's body weight was less than 14 g. We subsequently euthanized the mouse by CO_2 administration and submitted it for necropsy.

After receiving this mouse from the supplier (Jackson Laboratories, Bar Harbor, ME), we had housed it at a barrier facility in an individually ventilated polycarbonate microisolator cage (Thoren Caging Systems, Inc., Hazleton, PA). Husbandry procedures within the barrier facility included autoclaving of the microisolator cage after washing, supplying autoclaved bedding (ALPHA-dri, Shepherd, Watertown, TN) and autoclaved water bottles, and allowing *ad libitum* access to water and irradiated feed (7913; Harlan Teklad, Madison, WI). The animal was on study and was receiving intraperitoneal injections of corn oil. The animal technicians also reported that a cagemate of this animal was showing poor weight gain. We provide mice that become lethargic or cachexic during a study with Transgenic Dough Diet (Bio-Serv, Frenchtown, NJ) *ad libitum*. HEPA-filtered air at 15 air changes per hour supplied the room housing the mice; the cage system (Thoren) moved room air through a HEPA filter into the animal cages, and cage air pressure was positive with respect to the room. We monitored the specific pathogen-free status of the barrier facility by quarterly necropsy and comprehensive serology of sentinel animals exposed to used bedding, feed, and water bottles. Surveillance mice were negative by culture for murine bacterial pathogens and negative for *Helicobacter* spp. by PCR; we found no helminth or protozoal parasites in surveillance mice.

Gross necropsy of the cachectic female B6;129S2-*Tnfsf5*^{tm1Imx} mouse showed minimal contents to be present in the gastrointestinal tract and scant, hard feces present in the colon. Multiple tissue samples were fixed in Prefer (Anatech Ltd., Battle Creek, MI), a formalin-free fixative of glyoxal, in a buffered mixture of water and ethanol. We then submitted tissues for histopathological examination.

What do you expect the results of the histopathological examination will be? What do you think caused this mouse to become cachexic?

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What's your diagnosis?

Diagnosis:

Pneumocystis Pneumonia

Histopathological findings included marked, diffuse, granulomatous, interstitial pneumonia with exudative alveolitis, and intralesional organisms staining positive with Gomori's methenamine silver (GMS) that were morphologically consistent with *Pneumocystis carinii*. A thin rim of faint periodic acid–Schiff-positive (PAS-positive) staining makes these organisms visible microscopically (Fig. 1). GMS-stained sections demonstrated argyrophilic organisms of 5 μm diameter present in the alveolar exudate, consistent with *P. carinii* f. sp. *muris* (Fig. 2). The liver had mild, diffuse atrophy consistent with inanition. The mouse had mild, multifocal, granulomatous subcapsular pancreatitis consistent with intraperitoneal injections of corn oil. No significant lesions were found in the heart, spleen, or kidneys.

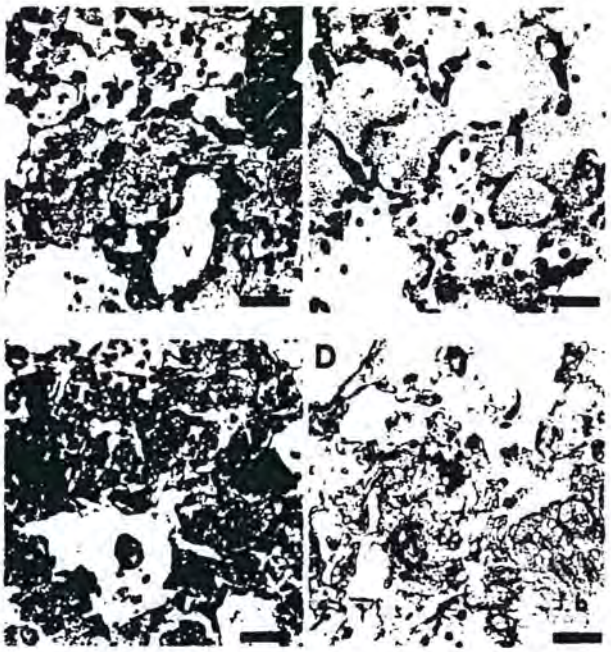

FIGURE 1. Histopathological alterations in the lung of a 16-week-old female B6;129S2-*Tnfsf5*^{tm1Imx} mouse with a history of low body weight. (A) H&E-stained section demonstrating inflammation and alveoli filled with foamy eosinophilic material containing tiny basophilic foci (bar = 30 μm). V, Blood vessel; b, bronchiole; f, alveolar filling. (B) Giemsa-stained section demonstrating alveolar filling (f) and myriads of organisms within alveoli (bar = 30 μm). (C) PAS-stained section demonstrating PAS-positive material filling alveoli (f) and surrounding clear spaces with faint blue dots that is consistent with *P. carinii* f. sp. *muris* (bar = 30 μm). (D) GMS-stained section showing argyrophilic cysts (arrows) present in the alveolar exudate, consistent with *P. carinii* f. sp. *muris* (bar = 30 μm). f, Alveolar filling.

Pneumocystis is a species-specific pathogen that can cause pneumonia in immunosuppressed or immunodeficient mammals. DNA analysis can differentiate the species of *Pneumocystis* that infects a specific host. *Pneumocystis* isolated from one host species cannot cause disease in a different host species. Host specificity has resulted in the recent classification of *Pneumocystis* organisms into two separate species. Microbiologists refer to the one that infects humans as *P. jiroveci*, and the one seen in rats as *P. carinii*. Using *P. carinii* as an umbrella term for the organism seen in all mammals is no longer acceptable. For example, when it is present in a mouse, we should differentiate the organism by calling it *P. carinii* special form *muris*[1].

Although *P. carinii* appears to be a fungus, it has evolved with a set of characteristics that make it a unique organism. Under light microscopy, it exhibits characteristics of both yeast and protozoa[1]. Its DNA sequence is similar to that of a fungus, but its RNA sequence is similar to that of yeast. The cyst form of the organism exhibits characteristics of a fungus. Its cyst wall contains chitin, and mitochondria are present. However, the trophic form, which is the primarily observed form of the organism, is yeastlike in appearance[1]. *Pneumocystis carinii* lacks ergosterol, the

FIGURE 2. A higher magnification of the GMS-stained section (Fig. 1D) demonstrating argyrophilic cysts present in the alveolar exudate, consistent with *P. carinii* f. sp. *muris* (bar = 10 μm).

LAB ANIMAL welcomes reader contributions to "What's Your Diagnosis?" in the case history/diagnosis format presented here; submissions should include two to five illustrations or photos. Please email your manuscript to labanimal@natureny.com or send a hard copy and an electronic copy on disk to Thomas M. Donnelly c/o Editorial Dept., LAB ANIMAL, 345 Park Avenue South, 10th Floor, New York, NY 10010. Selections are made on the basis of relevance and interest to readers.

primary sterol present in fungi[2], causing the organism to be resistant to antifungal drugs. However, antiprotozoan medications are often effective against *P. carinii* infection. Unlike most fungi, *P. carinii* has only one copy of the gene for ribosomal RNA and does not proliferate in culture[1]. Its exotic nature led Kaneshiro[2] to conclude that "*Pneumocystis* is *Pneumocystis.*"

In this case, the animal diagnosed with *P. carinii* f. sp. *muris* was a B6;129S2-*Tnfsf5*^{tm1Imx} mouse. This is a knockout strain lacking the CD40 ligand (CD40L), also known as CD154 and as tumor necrosis factor ligand superfamily, member 5. CD40L is expressed on $CD4^+$ and $CD8^+$ T cells[3], basophils[4], macrophages[5], and mast cells[6]. The interaction between CD40 and CD40L is essential to many pathways that are important to the proper functioning of the immune system. This interaction is necessary for the activation of helper T cells, and is therefore essential to the activation of B cells[7,8]. T cell–dependent activity of B cells ceases in the absence of CD40L. Therefore, Ig heavy chain isotype-class switching on immunoglobulins is unable to occur. Isotype switching is essential for the development of all isotypes except IgM[9], which is produced via a T cell–independent pathway. The CD40–CD40L interaction is necessary for both the activation of macrophages and the augmentation of cytokines, primarily interleukin-12 (ref. 5). This interaction also plays a role in the activation of dendritic cells, which are important antigen-presenting cells that activate T cells[10].

Mammals that lack the CD40L exhibit defects in both cellular and humoral immunity. This makes them susceptible to opportunistic infections such as *Pneumocystis spp.*[5] Although the CD40–CD40L interaction is essential to the resolution of disease caused by such organisms[11], it also seems to play a role in its pathogenesis[12]. CD40L is important to the inflammatory reaction and, therefore, the pulmonary injury that occurs in response to infection with *Pneumocystis spp.* ultimately increases the severity of the pneumonia. Other costimulatory pathways that play a role in the inflammatory reaction involve CD28 and ICAM1 (ref. 12).

CD40L knockout mice are an animal model for the human disease X-linked immunodeficiency with hyper IgM. Patients with this syndrome have a mutation in the CD40L gene[13]. As a result of the inability to undergo isotype-class switching[14], they lack IgG, IgA, and IgE, and show normal to elevated levels of IgM[15]. Besides the many conditions observed in patients with this disease, they are also more susceptible to opportunistic infections such as those associated with *P. jiroveci.* New treatment approaches presently being studied in CD40L knockout mice will hopefully be a step toward developing a treatment for patients with X-linked immunodeficiency with hyper IgM and other immune-suppressing diseases.

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