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Increased mortality from influenza infection in long-chain acyl-CoA dehydrogenase knockout mice

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ABSTRACT

We previously showed that the mitochondrial fatty acid oxidation enzyme long-chain acyl-CoA dehydrogenase (LCAD) is expressed in alveolar type II pneumocytes and that LCAD−/− mice have altered breathing mechanics and surfactant defects. Here, we hypothesized that LCAD−/− mice would be susceptible to influenza infection. Indeed, LCAD−/− mice demonstrated increased mortality following infection with 2009 pandemic influenza (A/CA/07/09). However, the mortality was not due to increased lung injury, as inflammatory cell counts, viral titers, and histology scores all showed non-significant trends toward milder injury in LCAD−/− mice. To confirm this, LCAD−/− were infected with a second, mouse-adapted H1N1 virus (A/PR/8/34), to which they responded with significantly less lung injury. While both strains become increasingly hypoglycemic over the first week post-infection, LCAD−/− mice lose body weight more rapidly than wild-type mice. Surprisingly, while acutely fasted LCAD−/− mice develop hepatic steatosis, influenza-infected LCAD−/− mice do not. They do, however, become more hypothermic than wild-type mice and demonstrate increased blood lactate values. We conclude that LCAD−/− mice succumb to influenza from bioenergetic starvation, likely due to increased reliance upon glucose for energy.

1. Introduction

Respiratory viral infections such as influenza have long been known to serve as triggers for metabolic decompensation and mortality in patients with genetic fatty acid oxidation (FAO) disorders [1]. The mechanisms behind the metabolic decompensation are not well understood. We previously showed that the alveolar type II pneumocyte (ATII), a key mitochondria-rich cell type in the lung, catalyzes FAO at high rates [2]. Both mouse and human ATII cells abundantly express the FAO enzyme long-chain acyl-CoA dehydrogenase (LCAD). LCAD−/− mice have increased lung epithelial permeability, altered breathing mechanics, and dysfunctional pulmonary surfactant [2]. Based on this, we hypothesized that LCAD−/− mice would show enhanced sensitivity to lung injury during a respiratory infection. To test this, LCAD−/− mice were infected with two different strains of influenza virus and evaluated for lung pathology as well as indicators of energy metabolism.

2. Methods

2.1. Animals and influenza virus treatments

All protocols were approved by the University of Pittsburgh Institutional Animal Care and Use Committee. LCAD ± mice (B6.129S6-*Acadl*^{tm1Uab}) were purchased from the Mutant Mouse Regional Resource Center (University of Missouri, Columbia, MO) on a C57Bl/6 strain background. Due to infertility on the C57Bl/6 background, the LCAD−/− mice used here were maintained on a mixed C57Bl/6 and 129S6 background. Age and gender-matched wild-type B6/129S6 mice served as controls for all experiments. For influenza virus infection, the mouse-adapted A/PR/8/34 H1N1 virus (PR8) was propagated in chicken eggs as described [3]. The 2009 pandemic virus A/CA07/09 H1N1 (CA07) was propagated in MDCK cells [4]. Female mice age 6–8 weeks were given 80 plaque forming units (pfu) in 50 μl of sterile PBS by oropharyngeal aspiration. Body weights were tracked daily. Blood glucose and lactate were measured in unrestrained animals by nicking the tip of the tail and collecting droplets of blood into assay strips for handheld analyzers. A digital rodent rectal temperature probe (Physitemps

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Instruments, Clifton, NJ) was used to monitor core body temperature.

2.2. Bronchoalveolar lavage fluid (BALF) collection and analysis

Mice were anesthetized, tracheotomized, and bronchoalveolar lavage performed using 1-ml of 0.9% NaCl. The amount of saline recovered was measured and recorded. The fluid was centrifuged at 300 × g for 10 min to pellet the cells, which were immediately resuspended in PBS, centrifuged onto glass slides, and stained for differential counting. The cell-free BALF supernatants were snap frozen in liquid nitrogen and stored at −80 °C for later viral titer assays performed by standard MDCK plaque assay [4].

2.3. Lung histology

The lungs were inflated with 10% neutral-buffered formalin at a pressure of 25 cm of H_2O for 10 min, then placed in fresh 10% neutral-buffered formalin for 24 h before processing for H&E staining. A pathologist blind to the genotypes scored lung injury on a severity scale of 1 (mild) to 5 (severe).

2.4. Liver triglycerides

Snap-frozen liver tissue was assayed for triglyceride content exactly as described [5].

2.5. Western blotting

Western blotting was carried out as described. Primary anti-surfactant protein-A (SP-A) antibody (Proteintech, Inc) was used at a dilution of 1:300.

3. Results & discussion

3.1. Increased mortality in LCAD−/− mice

Based on our previous work demonstrating altered breathing mechanics, surfactant defects, and increased epithelial permeability [2], we hypothesized that LCAD−/− mice would be more sensitive to influenza virus infection. Indeed, 8-week old LCAD−/− female mice infected with the CA07 virus displayed significantly reduced survival compared to wild-type control mice (Fig. 1A). LCAD−/− mice began to succumb by Day 5 post-infection compared to Day 8 for the wild-type controls. Weight loss is considered a good marker of disease severity in influenza-infected mice [6]; here, we observed significantly exacerbated weight loss in the LCAD−/− mice by Day 4 post-infection (Fig. 1B).

3.2. Increased mortality in LCAD−/− mice is not due to lung injury

Given the baseline lung defects previously established in LCAD−/− mice we hypothesized that the shortened survival time following infection was caused by enhanced lung injury. To test this, we repeated the CA07 infection but with tissue harvest on Day 5, just prior to the onset of mortality. The mice were subjected to bronchoalveolar lavage and lung tissue was processed for histopathology. Contrary to our hypothesis, the LCAD−/− mice did not show signs of enhanced lung injury following CA07 infection. There was a nonsignificant trend toward reduced inflammatory cells in bronchoalveolar lavage fluid (BALF) from LCAD−/− mice (Fig. 2A), and the distribution of these cell counts across neutrophils, monocytes, and lymphocytes were not different between genotypes (Fig. 2B). Equal titers of influenza virus in BALF indicated that viral replication and infectivity were not altered in LCAD−/− lungs (Fig. 2C). In keeping with the lack of difference in these parameters, histological examination of lung tissue revealed no significant difference in severity of injury as scored by a pathologist blind to the genotypes (Fig. 2D).

Seasonal H1N1 strains such as CA07 have been shown to cause less severe lung injury in mice than the more commonly used mouse-adapted PR8 strain [6,7]. We therefore infected a cohort of LCAD−/− and wild-type control mice with PR8. Compared to wild-type, LCAD−/− mice displayed significantly less lung tissue injury (Fig. 3A–C). The protection against injury in LCAD−/− lungs was limited to the parenchyma, as histology scores were not significantly different between genotypes in the perivascular or peribronchial regions of the lung.

The protection of LCAD−/− lung parenchyma from PR8-induced injury was an unexpected finding. Our previous work implicated surfactant defects as the cause of the altered breathing mechanics in LCAD−/− mice [2]. LCAD−/− had significantly less total surfactant as well as relative changes in composition in terms of acyl chain lengths and degree of unsaturation [2]. Surfactant is known to be the first line of defense against pathogens, but this defensive property is mostly ascribed to the surfactant proteins A (SP-A) and D (SP-D), which are innate immune system collectins that opsonize pathogens and mark them for phagocytosis by alveolar macrophages [8]. The lipid component of pulmonary surfactant has actually been shown to promote viral infection of the alveolar epithelium, particularly dipalmitoylphosphatidylcholine (DPPC), a highly abundant surfactant phospholipid with two saturated acyl

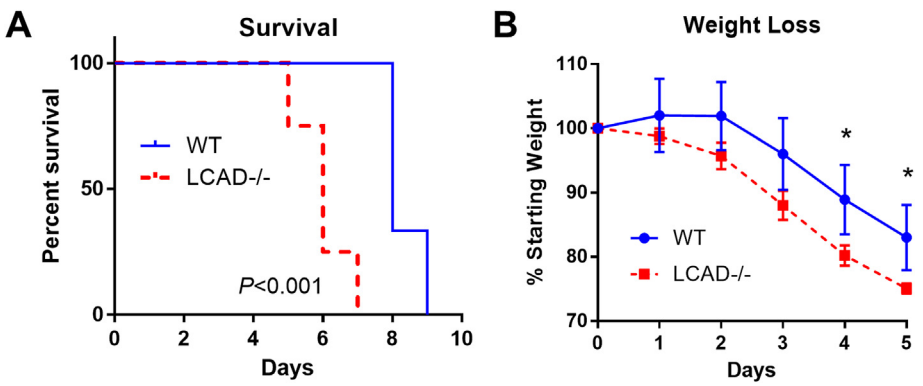

Fig. 1. Increased mortality in LCAD−/− mice following CA07 infection. LCAD−/− female mice (N = 6) and matched wild-type controls (N = 6) were infected with the 2009 pandemic CA07 influenza virus. A) LCAD−/− mice exhibited poorer survival and B) greater weight loss than control mice. *P < 0.05.

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