Original Article
OBESITY BIOLOGY AND INTEGRATED PHYSIOLOGY

Obesity

Diet-Induced Obese Mice Exhibit Altered Immune Responses to Acute Lung Injury Induced by *Escherichia coli*

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Objective: Obesity has been associated with impaired immunity and increased susceptibility to bacterial infection. It also exerts protective effects against mortality secondary to acute lung injury. The effects of obesity on immune responses to acute lung injury induced by *Escherichia coli* were investigated to determine if the above-mentioned differences in its effects were related to infection severity.
Methods: Diet-induced obesity (DIO) and lean control mice received intranasal instillations of 10^9 or 10^{10} CFUs of *E. coli*. The immune responses were examined at 0 h (uninfected), 24 h, and 96 h postinfection.
Results: Following infection, the DIO mice exhibited higher leukocyte, interleukin (IL)−10, IL-6, and tumor necrosis factor-α levels and more severe lung injury than the lean mice. Following inoculation with 10^{10} CFUs of *E. coli*, the DIO mice exhibited higher mortality and more severe inflammation-induced injury than the lean mice, but no differences in *E. coli* counts were noted between the two groups. However, inoculated with 10^9 CFUs of *E. coli*, the DIO mice exhibited smaller *E. coli* burdens at 24 h and 96 h after infection, as well as lower concentrations of IL-10 and tumor necrosis factor-α and less severe lung injury at 96 h after infection.
Conclusions: The results support the emerging view that obesity may be beneficial in the setting of milder infection but detrimental in the setting of more severe infection.

Obesity (2016) **24**, 2101-2110. doi:10.1002/oby.21608

Introduction

Globally, the numbers of individuals with obesity have reached alarming proportions. According to the latest estimates from the WHO, in 2014, more than 1.9 billion adults were overweight; of these, more than 600 million had obesity (11% of men and 15% of women) (1). Obesity is defined as abnormal or excessive fat accumulation that may impair health. Several comorbidities are associated with obesity, especially immune dysfunction, which results in alterations in immune cell function. Through various well-described pathophysiological mechanisms, obesity increases the risks of cardiovascular disease and other diseases, compromises quality of life, and increases overall mortality (2,3). Obesity is also recognized as a significant risk factor for pulmonary disease and is associated with elevated levels of circulating inflammatory cytokines and leukocytes (2,4), changes suggestive of a state of chronic systemic inflammation. Obesity dramatically influences lung responses to diseases such as asthma, chronic obstructive pulmonary disease, and chronic bronchitis (5).

Although many studies have found that obesity is strongly and disproportionately correlated with virus-associated hospitalizations and deaths (6,7), whether obesity is a risk factor for severe acute lung injury (ALI) secondary to bacterial infection remains unclear. Recent studies have demonstrated that obese leptin-deficient *ob*/*ob* mice exhibited impaired pulmonary bacterial clearance and defective alveolar macrophage phagocytosis and leukotriene synthesis; these mice exhibited increased susceptibility to infection and greater mortality following intratracheal challenges with either *Klebsiella pneumonia* or *Streptococcus pneumoniae* compared with lean WT mice (8,9). However, Hsu et al. (9) also confirmed that exogenous leptin administration enhanced the killing of bacteria by PMNs and improved pulmonary bacterial clearance and survival in *ob*/*ob* mice, suggesting that leptin influences host defenses against bacteria, not obesity. Preliminary clinical evidence indicates that an elevated BMI may exert protective effects against mortality secondary to community-acquired bacterial pneumonia and ventilator-associated ALI and also ameliorate ALI suggesting that obesity may influence disease courses and outcomes in patients with ALI (10), but the mechanisms underlying these effects are still unclear.

Previous studies have proven that obesity is associated with responses to lung infection, providing a basis to explore the relationship between obesity and ALI (11). *Streptococcus* is the most common cause of infection in patients with community-acquired

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Funding agencies: Natural Science Foundation of Science and Technology Department of Sichuan Province (2013NZ0032).
Disclosure: The authors declare no conflict of interest.
Received: 10 December 2015; **Accepted:** 11 May 2016; **Published online 25 August 2016. doi:10.1002/oby.21608**

pneumonia (12), and Gram-negative bacilli, such as *Escherichia coli*, frequently cause nosocomial pneumonia (13). Animals treated with intratracheal inoculations of specific amounts of *E. coli* would develop clinical ALI, according to Russo et al. (14). However, it is unclear whether recruitment of large numbers of immune cells, which contribute to both host defenses and inflammatory tissue injury and remodeling (15), is the main factor impacting host recovery after infection. In this study, we compared the inflammatory responses and pathologic lung injuries of lean mice and diet-induced obesity (DIO) mice, which exhibit changes similar to those observed in human patients with obesity, following intranasal challenges with different doses of *E. coli* to determine whether obesity affects the immune response to *E. coli* instillation and thus alters host defenses.

Methods

Animals

Three- to four-week-old male Kunming mice were purchased from Dashuo Animal Center (Chengdu, China) and housed under specific pathogen-free conditions. The animals were maintained according to specific protocols, and all euthanasia procedures were approved by the Guidelines for the Care and Use of Laboratory Animals and the Ethics Committee of Sichuan Agricultural University (Ya'an, China). The mice received either a normal diet or a high-fat diet for 8 weeks. During the experiment, food and water were supplied *ad libitum*, except during 8-h food deprivation periods before blood draws.

Diets

The diets, which have been described previously (16), were obtained from Dashuo Animal Center (Chengdu, China).

Organism

E. coli was obtained from the Sichuan Agricultural University Veterinary Medical Laboratory (Ya'an, China). The organisms were incubated at 37°C for 20 h in brain-heart infusion broth to obtain appropriate concentrations. Then the bacterial suspensions were centrifuged and suspended in sterile physiological saline (PBS) to produce the inoculums.

Respiratory tract infection

After 8 weeks on the above-mentioned diets, the mice were anesthetized with ether and challenged intranasally with 40 μL of a bacterial suspension containing approximately 10^9 or 10^{10} colony-forming units (CFUs) of *E. coli* diluted in PBS via a sterile 24-gauge needle. Preliminary studies from our laboratory determined that 10^9 CFUs of *E. coli* was sufficient to elicit an immune response but do not cause mortality in either the obese or the control mice and that 10^{10} CFUs of *E. coli* was unlikely to cause significant mortality in either group of mice.

Preparation of serum

The mice were bled retro-orbitally after receiving their respective diets for 8 weeks. Individual sera were separated from clotted blood via centrifugation and stored at −80°C until cytokine assays were performed.

Preparation of bronchoalveolar lavage and cell counting

At predetermined times after infection, i.e., time 0 (preinfection) and 24 h and 96 h postinfection, bronchoalveolar lavage (BAL) samples were obtained by injecting and aspirating 0.4 mL of PBS through the trachea. This procedure was repeated three times per mouse. The BAL fluid (BALF) samples were pooled, and the cells were isolated via centrifugation at 1,500 rpm for 10 min and resuspended in 100 μL of PBS. BALF cell counts were determined using an automatic blood cell counter (ABACUS Junior Vet, Switzerland).

Preparation of lung homogenates

At various times after infection, the apical and intermediate lobes of the right lungs were harvested and homogenized in 1 mL of PBS using an Ultra-turrax Tissue Homogenizer. The homogenates were then centrifuged at 2,000 rpm for 10 min at 4°C. The supernatants were passed through a 0.45 μm filter, collected, and then stored at −80°C for cytokine production analysis.

Cytokine assays

Tumor necrosis factor (TNF)-α, interleukin (IL)−6, and IL-10 concentrations in the serum and lung homogenate supernatants of infected mice were measured with mouse ELISA kits (R&D Systems, China), according to the manufacturer's instructions.

Determination of wet/dry ratios

To diagnose pulmonary edema, the wet/dry ratios of the lungs were measured. After the mice were euthanized, representative tissue samples were taken from the inferior lobes of the left and right lung and then weighed. The samples were weighed again after 24 h of drying at 65°C, which represented their baseline dry weight.

Lung histopathology

For histopathologic analysis, the upper lobes of the left lungs were removed and immediately fixed in 10% neutral buffered formalin. Lung sections (5 μm) were taken beginning at 100μm from a designated reference point and collected at 100μm intervals, and three sections of the left lung from each animal were stained with hematoxylin and eosin (H&E). Then, the sections were visualized by light microscopy.

Bacteriological examination

The lungs were removed aseptically from the sacrificed mice and homogenized with 1 mL of PBS, using an Ultra-turrax Tissue Homogenizer. The homogenates were serially diluted 10-fold with PBS, and 10 μL samples of these dilutions were inoculated onto MacC agar plates. The plates were incubated at 37°C for 20 h. Colonies were enumerated, and the bacterial counts in the lungs were expressed as the log number of CFUs per lung.

Statistical analysis

All data are expressed as the mean ± SD of three independent experiments. Statistical analyses were performed to compare the obese groups with the lean groups via one-way analysis of variance, followed by the Tukey-Kramer multiple comparison test. All

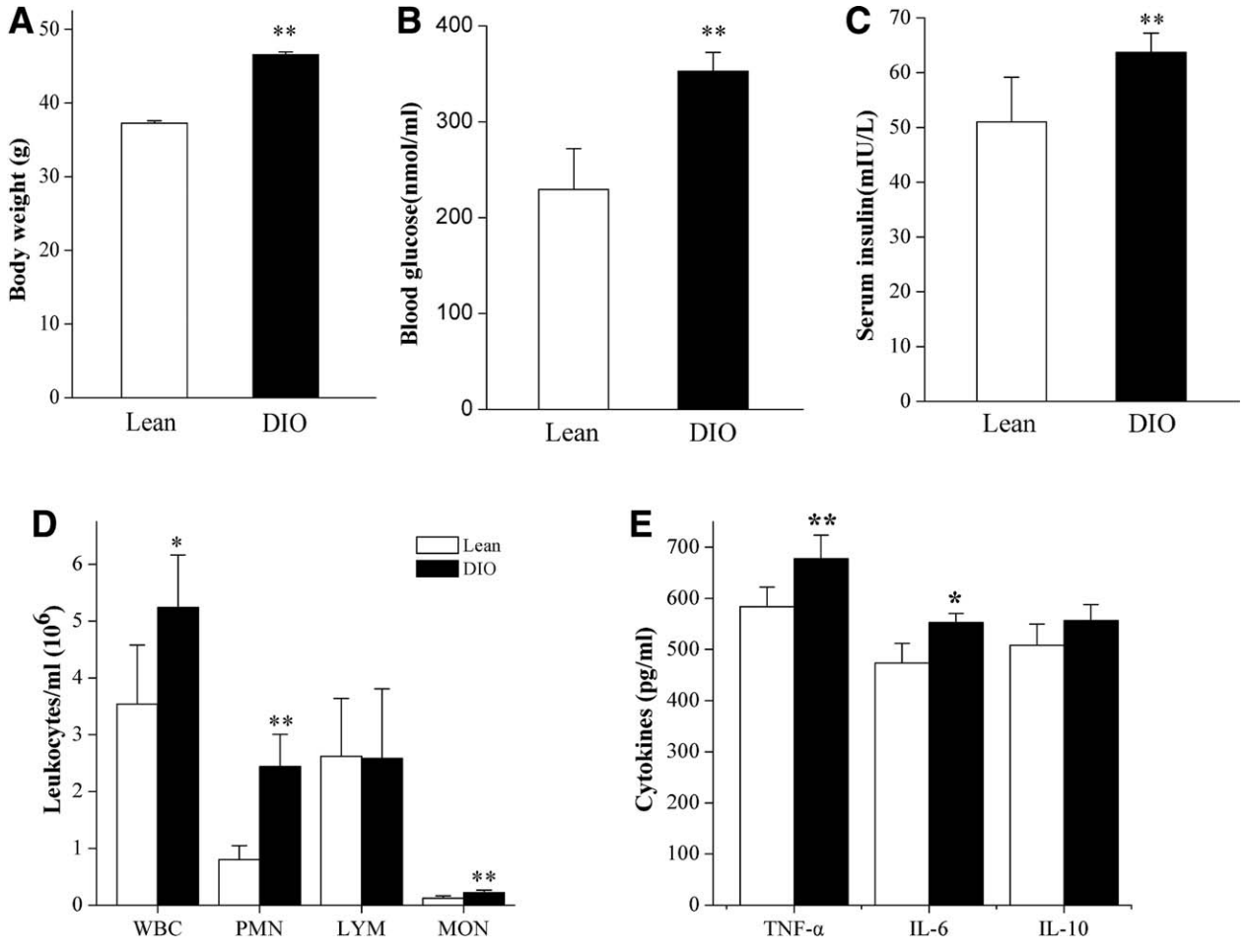

Figure 1 (**A**) Body weights, (**B**) blood glucose levels, (**C**) serum insulin levels, (**D**) white blood cell counts, and (**E**) serum tumor necrosis factor (TNF)-α, interleukin (IL)-26, and IL-10 levels, at baseline in mice fed a high-fat diet or a regular diet for 8 weeks. Values are expressed as the mean ± SD, $n = 6$–8 mice per group; asterisks indicate significant differences between the diet-induced obesity (DIO) mice and the lean control mice (*$P < 0.05$, **$P < 0.01$).

statistical analyses were performed using a commercially available statistical software package (SPSS17.0, SPSS Inc.).

Results

Metabolic abnormalities and immune disorders in DIO mice

All of the mice had similar body weights at the outset of the study (data not shown). However, after being fed *ad libitum* for 8 weeks, the mice maintained on a high-fat diet were significantly heavier than the mice maintained on a regular diet. In addition, the DIO mice were hyperglycemic and exhibited substantially higher serum insulin levels than their counterparts. The DIO mice exhibited higher total white blood cell counts than the lean controls, which were attributed to elevated numbers of neutrophils (PMN) and monocytes rather than lymphocytes, as well as higher serum concentrations of IL-6 and TNF-α and modestly elevated IL-10 levels (shown in Figure 1).

Higher mortality in DIO mice following *E. coli* infection

No deaths were noted in either the DIO or the lean mice when the groups were challenged intranasally with 10^9 CFUs of *E. coli*. However, after being challenged with 10^{10} CFUs of *E. coli*, several DIO and lean mice died within 12 h. The remaining lean mice survived until 72 h postinfection, while the remaining DIO mice survived until 96 h postinfection. Then, no mice died in either group for 1 week (data not shown). The mortality rates in both groups at 24 h and 48 h postinfection were higher than those at any other times. The total mortality rate was higher among the DIO mice than among the lean mice (shown in Figure 2).

Differences in weight loss and bacterial burdens in mice following *E. coli* infection

As shown in Figure 3, the lean mice that were challenged intranasally with *E. coli* exhibited gradual increases in body weight loss, and the DIO mice exhibited greater total weight loss at 72 h postinfection with the same bacterial dose. This weight loss recovered over a 96-h period

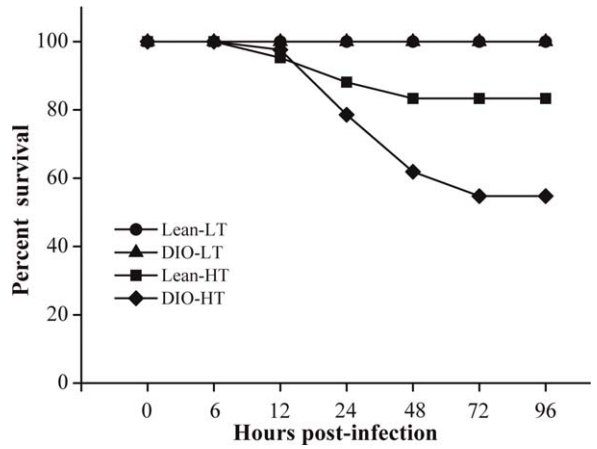

Figure 2 Survival percentages of lean and diet-induced obesity (DIO) mice following intranasal challenges with different doses of *E. coli*. The survival percentages of lean mice administered 10^9 CFUs of *E. coli* (lean-LT) or 10^{10} CFUs of *E. coli* (lean-HT) and those of DIO mice administered 10^9 CFUs of *E. coli* (DIO-LT) or 10^{10} CFUs of *E. coli* (DIO-HT) were calculated over a 96-h period. $n = 16–20$ per group.

in all four groups, especially in the DIO mice challenged with 10^9 CFUs of *E. coli*, but worsened in the mice challenged with 10^{10} CFUs of *E. coli*. Following treatment with 10^{10} CFUs of *E. coli*, the weight loss and recovery were attributed mainly to changes in food intake elicited by *E. coli* between 6 h and 72 h after infection (data not shown). To determine whether increased mortality and weight loss were associated with impaired bacterial clearance in the DIO mice, we measured lung bacterial loads at 24 h and 96 h postinfection. Compared with the lean controls, we found that the DIO mice exhibited significantly smaller bacterial loads at 24 h and 96 h after treatment with 10^9 CFUs of *E. coli*; however, the difference between the two groups did not reach statistical significance following treatment with 10^{10} CFUs of *E. coli*.

Increased numbers of immune cells in the BALF of DIO mice following *E. coli* infection

After treatment with 10^9 or 10^{10} CFUs of *E. coli*, both total and differential leukocyte counts were measured to determine whether changes in these parameters were associated with lung injury and recovery in obese mice. Similar to previous findings, increased baseline lung leukocyte counts were noted in the DIO mice compared with the lean mice. After challenge, we found that the levels of all leukocytes subsets (including monocytes, lymphocytes, and neutrophils) were significantly increased in the BALF of both the DIO and lean mice but decreased by 96 h postinfection. Compared with the lean controls, the DIO mice exhibited higher leukocyte counts in response to the same bacterial challenge. It should be noted that the majority of leukocytes in the BALF were neutrophils. Although the difference in overall cell numbers between the DIO and lean mice was statistically significant, the patterns of the changes caused by *E. coli* administration were similar between the two groups (shown in Figure 4).

Effect of *E. coli* instillation on cytokine levels in lung homogenates

Excess adipose tissue has been shown to produce large numbers of cytokines, which cause chronic low-grade systemic inflammation in humans and animals with obesity (17,18). In our study, the concentrations of TNF-α, IL-6, and IL-10 in lung homogenates were assessed before infection and at 24 h and 96 h post-*E. coli* administration. As shown in Figure 5, the DIO mice exhibited elevated lung homogenate TNF-α, IL-6, and IL-10 concentrations at baseline. Production of these cytokines increased significantly in response to *E. coli* administration in both the obese and lean mice. Compared with the lean animals, the DIO mice exhibited significantly higher levels of TNF-α, IL-6, and IL-10 following inoculation with the same dose of *E. coli* at 24 h postinfection. Additionally, the increases in cytokine levels were positively correlated with the dose of *E. coli*. At 96 h postinfection, the levels of all cytokines decreased more quickly in the obese mice than in the lean mice. Although the concentrations of TNF-α, IL-6, and IL-10 remained higher in the DIO mice challenged with 10^{10} CFUs of *E. coli*, the concentrations were lower in the DIO mice challenged with 10^9 CFUs of *E. coli*.

Effect of obesity on lung wet/dry ratios after *E. coli* infection

Some studies have reported that obesity impairs pulmonary vascular homeostasis and enhances susceptibility to acute injury by altering the expression of cell adhesion molecules in the pulmonary vascular endothelium and disrupting endothelial cell barrier function (19,20).Tissue damage caused by infection was evaluated via wet-to-dry weight (*W/D*) ratios in this study. Our results showed that the basal *W/D* ratios were similar between the uninfected DIO and control mice. Twenty-four hours after infection, the *W/D* ratio increased significantly in the DIO mice and increased modestly in the control mice. However, 96 h after administration, the *W/D* ratio decreased sharply and was lowest in the DIO mice infected with 10^9 CFUs of *E. coli* and highest in the DIO mice infected with 10^{10} CFUs of *E. coli* (shown in Figure 6).

Differences in histopathology following *E. coli* infection

Histological evaluations of lung injury severity were performed on lung tissue samples that were obtained from subgroups of mice. We observed normal alveolar architecture in the uninfected DIO and lean mice, in which a single layer of pneumocytes was observed. Following infection, the severity of tissue damage was positively correlated with the dose of *E. coli* administered. As early as 6 h after infection, immune cells were observed in the lungs of the infected animals, and the DIO mice exhibited significantly higher numbers of immune cells than their counterparts. At 24 h after infection, significant alveolar destruction had occurred in the infected animals, as well as infiltration of the alveoli, bronchi, and lung parenchyma by large numbers of immune cells. Immune cells were observed throughout the lungs of the DIO mice that were challenged with 10^{10} CFUs of *E. coli*. At 72 h after infection, the number of immune cells had significantly decreased, and the reemergence of air-filled spaces was evident. At 96 h after infection, significant air space restoration had occurred; however, the alveolar septae remained thickened. As shown in Figure 7, at 72 h and 96 h postinfection, the DIO mice that were inoculated with 10^9 CFUs of *E. coli* exhibited fewer immune cells and exudates in both the lung parenchyma and the alveoli than the other groups of infected mice.

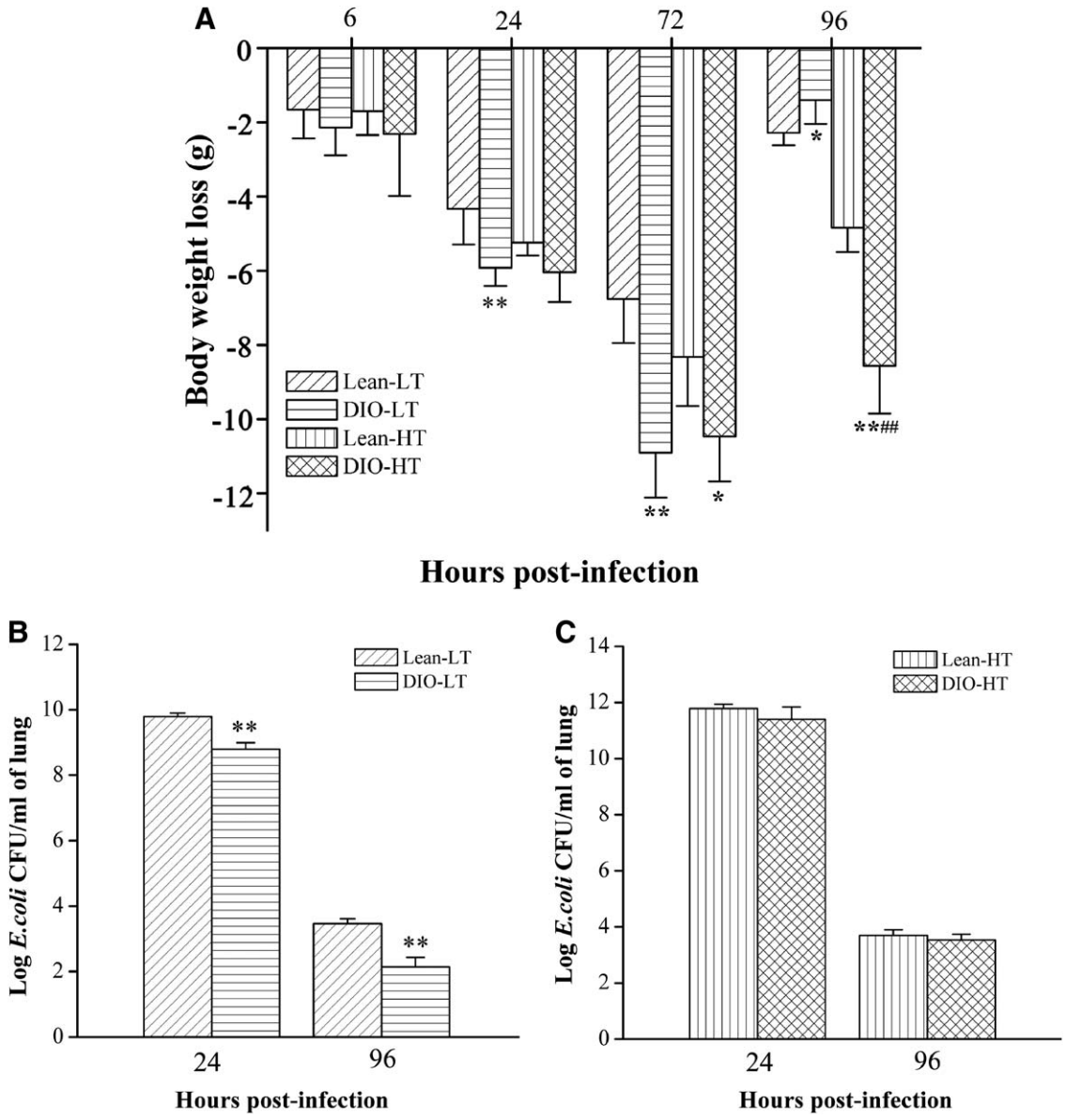

Figure 3 The effects of *E. coli* infection on body weight loss and lung bacterial burden in lean and diet-induced obesity (DIO) mice. (**A**) Body weight loss, expressed as total weight loss in lean mice that were infected intranasally with 10^9 CFUs of *E. coli* (lean-LT) or 10^{10} CFUs of *E. coli* (lean-HT) and in DIO mice that were infected intranasally with 10^9 CFUs of *E. coli* (DIO-LT) or 10^{10} CFUs of *E. coli* (DIO-HT), was measured at 6, 24, 72, and 96 h postinfection. Bacterial counts in the lung homogenates of the (**B**) DIO-LT and lean-LT and (**C**) DIO-HT and lean-HT groups were assessed at 24 and 96 h post-*E. coli* challenge. Values are expressed as the mean ± SD, $n = 3$–4 mice per group at each time point. Asterisks indicate differences from the lean controls that received the same treatment at the indicated time points: $^*P < 0.05$,$^{**}P < 0.01$; octothorps indicate differences from the lean or obese controls versus those infected with 10^9 CFUs of *E. coli* at the indicated time points: $^{\#\#}P < 0.01$.

Discussion

There have been numerous reports regarding populations with obesity exhibiting increased susceptibility to bacterial infection, as well as more severe illness and death secondary to lung injury caused by bacterial infection (21,22). Recently, large cohort studies have shown that obesity may exert protective effects against ALI and community-acquired pneumonia, indicating that clinically significant alterations in the acute pulmonary inflammatory response may be associated with weight gain. Because of the increasing numbers of individuals with obesity worldwide, it is critical to determine how obesity impacts the host's ability to respond to lung injury. Moreover, limited numbers of rodent models have been used to study the impact of excess adiposity on immune function and host defenses against infection, and the majority of these models have involved genetically obese mice, such as db/db and ob/ob mice. Few studies have utilized the DIO model in the context of ALI. DIO is a more physiologically relevant model of human obesity, as only a small number of individuals with obesity have mutations in the leptin

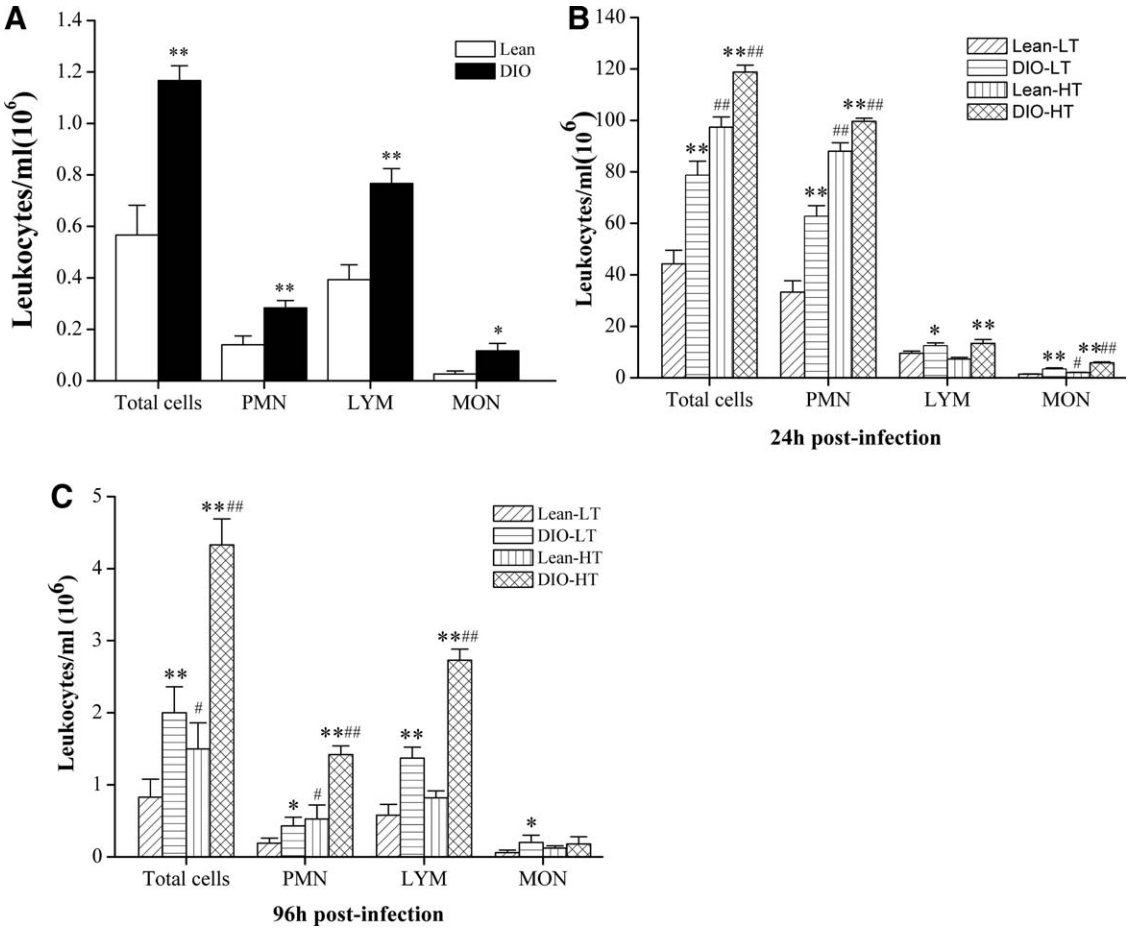

Figure 4 Effects of diet-induced obesity (DIO) on immune cell recruitment to bronchoalveolar lavage fluid (BALF) following intranasal infection with *E. coli*. Total cell and neutrophil (PMN), lymphocyte (LYM), and monocyte (MON) counts were measured in the BALF of (**A**) uninfected lean and DIO mice and of lean mice infected with 10^9 CFUs of *E. coli* (lean-LT) or 10^{10} CFUs of *E. coli* (lean-HT) and DIO mice infected with 10^9 CFUs of *E. coli* (DIO-LT) or 10^{10} CFUs of *E. coli* (DIO-HT) at (**B**) 24 h and (**C**) 96 h postinfection. Values are expressed as the mean ± SD, $n = 4$–5 mice per group at each time point. Asterisks indicate differences between the DIO and lean mice that received the same treatment at the indicated time points: $^{*}P < 0.05$, $^{**}P < 0.01$; octothorps indicate lean or DIO mice versus 10^9 CFUs of *E. coli* at the indicated time points: $^{\#}P < 0.05$, $^{\#\#}P < 0.01$.

gene. In this article, we established a Kunming mouse obesity model by administering a high-fat diet for 8 weeks, which has been described previously (23,24).The DIO mice were heavier than the control mice and exhibited higher serum levels of insulin, glucose, IL-6, and TNF-α, as well as higher peripheral blood leukocyte counts, findings consistent with those of other reports (25,26). These alterations appear to cause a state of chronic systemic inflammation, which influences immune function and the responses of the lung to acute infection (2). In this study, for the first time, we compared the responses of lean mice and DIO mice following intranasal administration of different doses of *E. coli* to determine the role of obesity in Gram-negative bacteria-induced lung injury.

Lawrence et al. (27) found that both DIO and ob/ob obese mice displayed altered behavioral responses and cytokine release patterns in response to systemic inflammation induced by intraperitoneal administration of LPS. Our data demonstrated that DIO animals experience greater body weight loss after *E. coli* treatment than control animals and that animals treated with higher doses of *E. coli* take longer to recover than animals treated with lower doses; however, contrasting findings were noted in the 10^9 CFUs dose group. The changes in body weight in the mice with DIO were attributed to reductions in food intake, particularly after infection with high doses of bacteria (27).Although it has been reported that host defenses may be impaired in other models of obesity, resulting in a reduced capacity to clear bacteria (8), in the model used in this study, total lung bacterial titers decreased significantly in the obese mice after inoculation with 10^9 CFUs of *E. coli*, a finding similar to that observed in $CPE^{fat/fat}$ mice with pneumococcal infection (28).However, the pulmonary bacterial burdens in the DIO mice were not different from those in the control mice challenged with 10^{10} CFUs of *E. coli*.

Adipocytes can produce many cytokines, such as IL-6, IL-8, and TNF-α, and the levels of these cytokines are elevated in the peripheral blood of individuals with obesity (29). It has also been confirmed that enhanced pulmonary inflammatory responses can occur due to increased cytokine and neutrophil recruitment in the lungs of

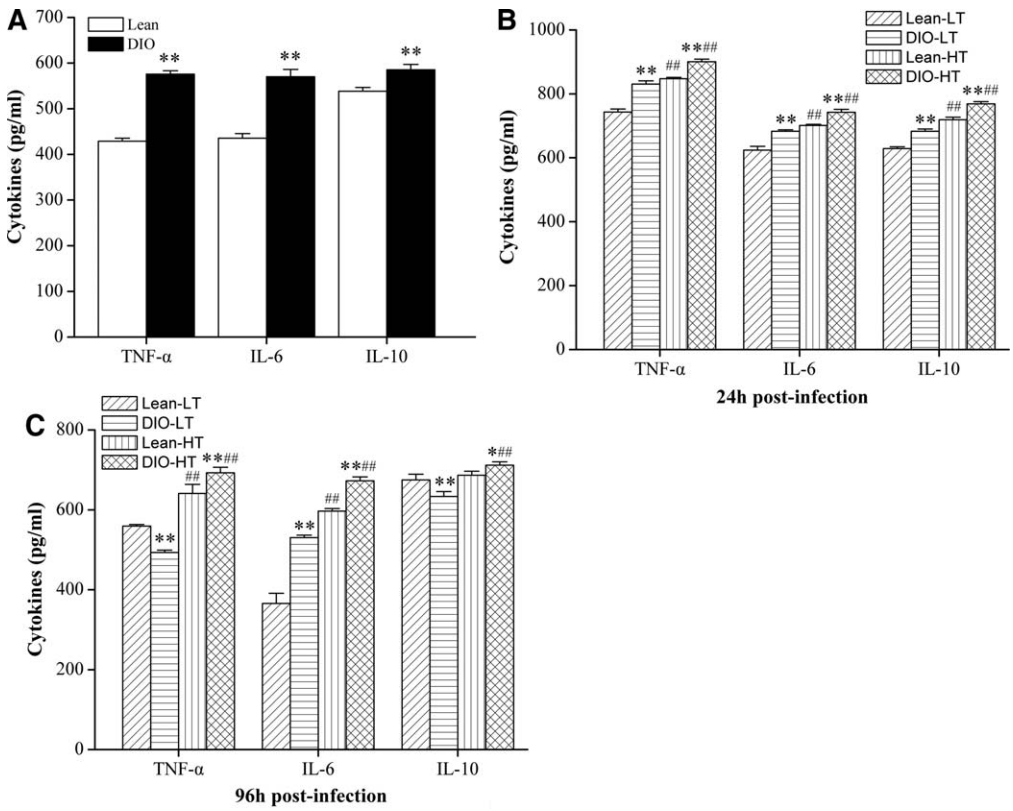

Figure 5 Cytokines in the lung homogenates of diet-induced obesity (DIO) mice and lean mice. Tumor necrosis factor (TNF)-α, interleukin (IL)-26, and IL-10 levels were measured in the lung homogenates of (**A**) uninfected lean and DIO mice and of lean mice intranasally infected with 10^9 CFUs of *E. coli* (lean-LT) or 10^{10} CFUs of *E. coli* (lean-HT) and DIO mice intranasally infected with 10^9 CFUs of *E. coli* (DIO-LT) or 10^{10} CFUs of *E. coli* (DIO-HT) at (**B**) 24 h and (**C**) 96 h postinfection. Values are expressed as the mean ± SD, $n = 4$–5 mice per group at each time point. Asterisks indicate differences between the DIO and lean mice that received the same treatment at the indicated time points: $^*P < 0.05$, $^{**}P < 0.01$; octothorps indicate lean or DIO control mice versus those infected with10^9 CFUs of *E. coli* at the indicated time points: $^{\#\#}P < 0.01$.

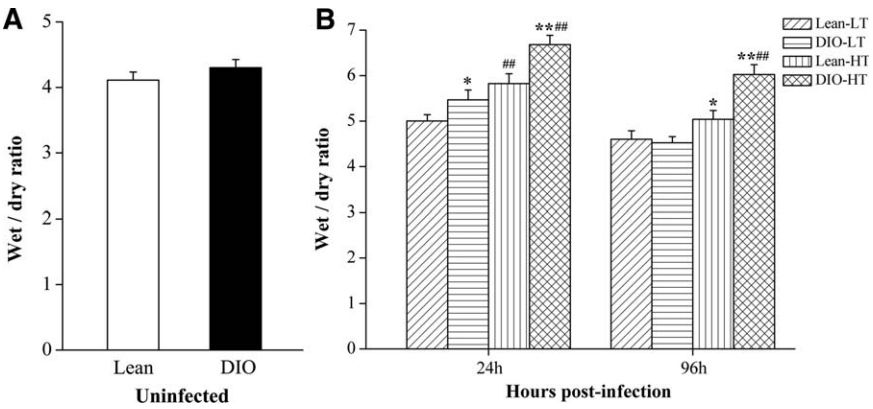

Figure 6 Lung wet-to-dry weight (*W/D*) ratios in diet-induced obesity (DIO) mice and lean mice with or without *E. coli* treatment. *W/D* ratios were measured (**A**) in uninfected DIO and lean mice and (**B**) in lean mice infected with 10^9 CFUs of *E. coli* (lean-LT) or 10^{10} CFUs of *E. coli* (lean-HT) and DIO mice infected with 10^9 CFUs of *E. coli* (DIO-LT) or 10^{10} CFUs of *E. coli* (DIO-HT) at 24 h and 96 h postinfection. Values are expressed as the mean ± SD, $n = 4$–5 mice per group at each time point. Asterisks indicate differences between the DIO and lean mice that received the same treatment at the indicated time points: $^*P < 0.05$, $^{**}P < 0.01$; octothorps indicate lean or DIO control mice versus those infected with10^9 CFUs of *E. coli* at the indicated time points: $^{\#\#}P < 0.01$.

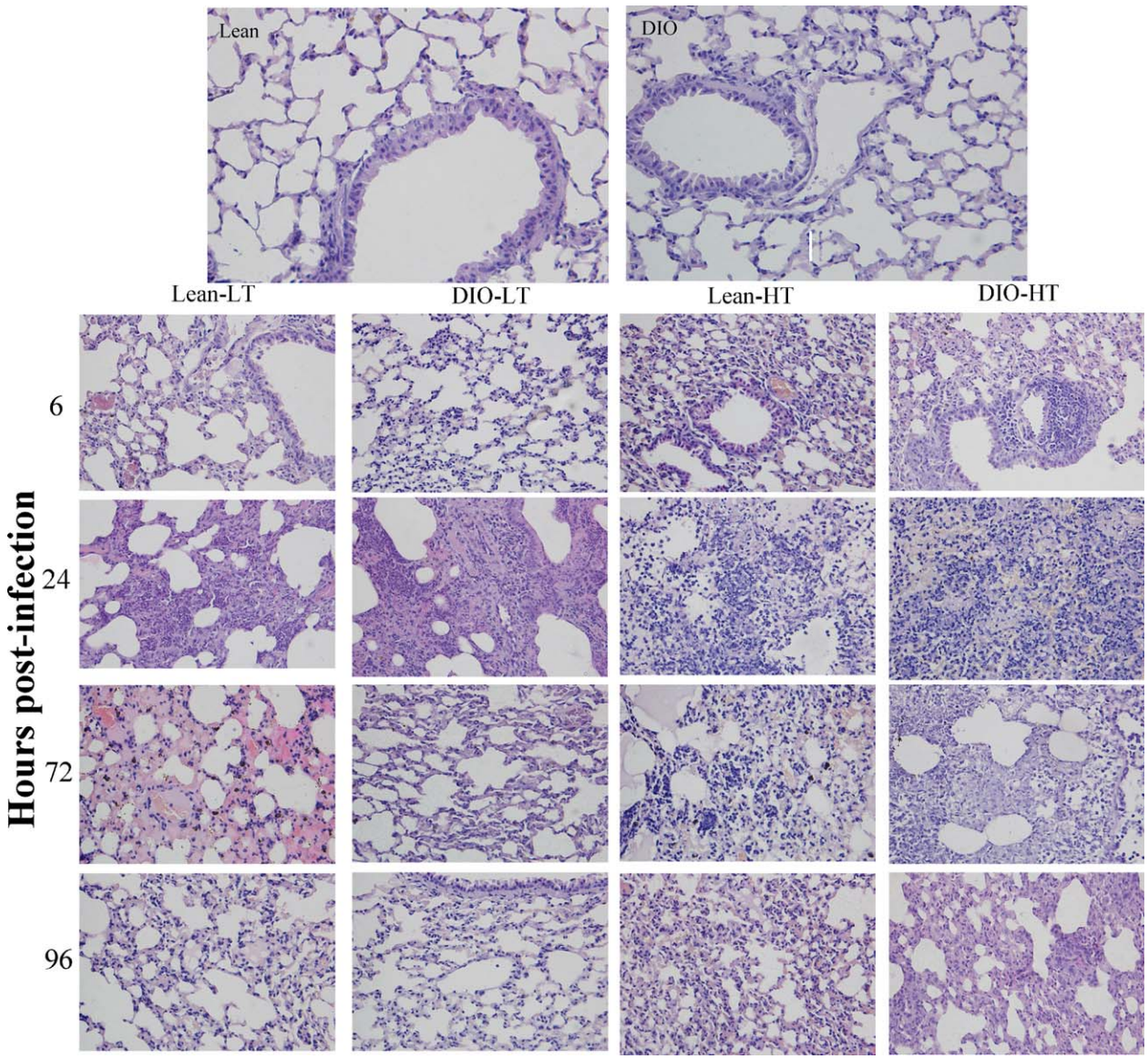

Figure 7 Inflammatory responses to administration of 10^9 or 10^{10} CFUs of *E. coli* in lean and diet-induced obesity (DIO) mice. Hematoxylin and eosin (H&E) staining of lung sections was visualized via light microscopy to examine lung architecture and immune cell infiltration. Images were taken at 400× magnification. Histopathological results were analyzed in lean mice infected intranasally with 10^9 CFUs of *E. coli* (lean-LT) or 10^{10} CFUs of *E. coli* (lean-HT) and DIO mice intranasally infected with 10^9 CFUs of *E. coli* (DIO-LT) or 10^{10} CFUs of *E. coli* (DIO-HT) in the absence of infection and 6, 24, 72, and 96 h postinfection. $n = 4$–5 mice per group at each time point. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

genetically obese mice (9,30); however, there are few reports regarding animals with DIO. In this study, we measured the concentrations of various cytokines in lung homogenates. Although DIO mice have exhibited significant differences in cytokine release in the lungs compared with db/db obese mice following acute lung injury (31), that the DIO mice studied here exhibited higher IL-6, IL-10, and TNF-α levels in their lung homogenates after *E. coli* instillation, findings similar to those involving ob/ob and $CPE^{fat/fat}$ mice (30). At 96 h postinfection, the levels of IL-10 and TNF-α in the lungs of the DIO mice were significantly lower than those in the lungs of the control mice; however, IL-6 levels remained higher in the DIO mice than in the lean controls challenged with 10^9 CFUs of *E. coli*. Following administration of 10^{10} CFUs of *E. coli*, the obese mice exhibited persistently elevated cytokine levels; the mechanism underlying these effects remains unknown. Kordonowy et al. (31) noted that ALI is attenuated in obese mice and that this blunted response is partially attributable to obesity-related abnormalities in neutrophil chemoattractant responses. Although plasma IL-6 levels were decreased in the obese mice in our study compared with the control mice in the setting of established lung injury, no differences in airspace inflammatory cytokine levels were noted between the obese and lean mice following acute lung injury.

We and others have observed that obese mice appear to exhibit significant alterations in leukocyte migration to the lungs after infection, particularly neutrophils (9,32).These abnormalities may be

attributed to obesity-induced impairments in pulmonary vascular homeostasis and enhanced susceptibility to acute injury (33). Neutrophils are known to ingest and kill bacteria, and elevated levels of PMNs may facilitate enhancements in host defenses against bacterial infections, contributing to host recovery. Xu et al. (34) reported that African American males with obesity exhibit increased neutrophil percentages and activity and suggested that neutrophils play an essential role in the pathogenesis of obesity-related diseases. However, PMN activation and migration to the lungs in certain pathological states also contribute to inflammatory tissue injury and tissue architecture remodeling (15). Neutrophils contain enough cytotoxic and proteolytic materials to induce lesional changes, and significant recruitment of these cells to sites of infection may induce collateral tissue damage by activating proteases that degrade the extracellular matrix of target tissues, which may diminish bacterial clearance (35) and contribute to global lung injury after *E. coli* infection. In our study, we observed that obese mice exhibited faster and greater immune cell recruitment than lean control mice post-*E. coli* infection; however, at 96 h postinfection in the 10^9 CFUs groups, the lean control mice exhibited more severe lung injury than their counterparts. Among the groups administered 10^{10} CFUs of *E. coli*, the DIO mice exhibited greater numbers of immune cells in the lung parenchyma and alveoli at 96 h postinfection than their counterparts, findings similar to those noted by Grewal et al. (36). These differences may be associated with differences in animal models and infection severity. Fujiwara et al. (37) found that administration of a lard-based HFD for 12 weeks attenuated LPS-induced ALI via increased pulmonary SLPI expression in rats, which did not occur after 4 weeks of administration of the same diet. Infection can stimulate almost all types of leukocytes, which cooperate to control and eradiate pathogens. In the setting of severe infection, the majority of leukocytes exhibit functional alterations, which may contribute to the secondary effects exerted by a relative excess of antigen or particulate debris (38).These factors may explain why the DIO mice exhibited greater mortality following *E. coli* infection than their counterparts, as well as why similar findings have been noted in studies involving other obese animals treated with influenza virus and other bacteria (27,39). The data in this study indicated that DIO may exert contrasting effects on host recovery from acute lung injury.

In summary, clinical studies indicate that obesity is a significant risk factor for development of ALI; however, obesity also exerts protective effects that ensure survival and improve outcomes in patients with pneumonia and ALI. The mechanisms underlying these effects are not well understood. For the first time, we investigated the impact of DIO on the immune response to infection and host defenses against different doses of *E. coli*, and we demonstrated that obesity elicited contrasting responses to ALI. During milder infections, obesity improved host defenses against infection, promoting recovery. However, in the setting of more severe infection, obesity exerted negative effects on host defenses. These findings will likely advance our understanding of the pathogenesis of ALI while also facilitating development of novel therapies to prevent and treat this disease.O

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