MAJOR ARTICLE

Leptin Mediates the Pathogenesis of Severe 2009 Pandemic Influenza A(H1N1) Infection Associated With Cytokine Dysregulation in Mice With Diet-Induced Obesity

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Background. Obesity is associated with a high circulating leptin level and severe 2009 pandemic influenza A virus subtype H1N1 (A[H1N1]pdm09) infection. The mechanism for severe lung injury in obese patients and the specific treatment strategy remain elusive.

Method. We studied the pathogenesis of A(H1N1)pdm09 infection in a mouse model of diet-induced obesity.

Results. Obese mice had significantly higher initial pulmonary viral titer and mortality after challenge with A(H1N1)pdm09, compared with age-matched lean mice. Compared with lean mice, obese mice had heightened proinflammatory cytokine and chemokine levels and more severe pulmonary inflammatory damage. Furthermore, obese mice had a higher preexisting serum leptin level but a lower preexisting adiponectin level. Recombinant mouse leptin increased the interleukin 6 (IL-6) messenger RNA expression in mouse single-lung-cell preparations, mouse macrophages, and mouse lung epithelial cell lines infected with A(H1N1)pdm09. Administration of anti-leptin antibody improved the survival of infected obese mice, with associated reductions in pulmonary levels of the proinflammatory cytokines IL-6 and interleukin 1β but not the pulmonary viral titer.

Conclusions. Our findings suggest that preexisting high levels of circulating leptin contribute to the development of severe lung injury by A(H1N1)pdm09 in mice with diet-induced obesity. The therapeutic strategy of leptin neutralization for the reduction of proinflammatory responses and pulmonary damage in obese patients warrants further investigations.

Keywords. obesity; leptin; adiponectin; mice; influenza; A(H1N1)pdm09; severe.

Obesity is a global health threat [1]. Besides its well-established associations with type II diabetes mellitus, cardiovascular diseases, and liver diseases [2], obesity is also a risk factor for infectious diseases [3, 4]. Multiple factors, including immunological changes, have been postulated to explain the increased susceptibility to infection among obese patients, but the exact mechanism is still uncertain [4]. During the 2009 influenza pandemic, obesity, in addition to other risk factors such as extremes of age, pregnancy, underlying comorbidities, immunoglobulin G2 deficiency, and genetic polymorphisms, increased the likelihood of hospitalization, admission to the intensive care unit, and death [5–9]. Although pulmonary function is impaired in obese patients [10], the mechanism underlying the pathogenesis specific to severe influenza in these patients remains elusive. Studies in obese mouse models suggested that both innate and adaptive immune responses to influenza A virus and its vaccine antigens, such as the type I interferon response, natural killer cell function, antigen presentation by dendritic cells, and antigen-specific memory of CD8+...
lymphocytes, were defective [11–15]. Impaired wound repair and associated pulmonary edema have been observed in severe lung damage due to influenza virus [16]. Even vitamin D deficiency associated with obesity was postulated to be linked with severe disease [17]. Since the mechanism leading to severe influenza in obese patients is still unknown, there is currently no specific treatment for these patients. In this study, we used a mouse model of diet-induced obesity to investigate the contributions of the circulating adipokines leptin and adiponectin in the pathogenesis of severe influenza virus infection in an obese host.

**MATERIALS AND METHODS**

**Virus Strains**
HK/415742/09 (A[H1N1]pdm09), the wild-type human strain of 2009 pandemic influenza A virus subtype H1N1, and HK/415742/09-Mut (pdmH1N1-Mut), the mouse-adapted mutant strain of pdmH1N1 [18, 19], were propagated in 10-day-old specific-pathogen-free embryonic eggs. Allantoic fluid was harvested and titrated on a Madin-Darby canine kidney cell monolayer. Aliquots of virus stock were frozen at −80°C for later use [18, 19].

**Obesity Induction in C57BL/6N Mice**
Obesity induction was performed as described previously [20]. Three-week-old weanling female C57BL/6N mice were divided randomly into 2 groups. One group was fed a high-fat diet containing 45% Kcal from fat (PicoLab Rodent Diet 20, LabDiet Code 5053, PMI). The body weight of each mouse was monitored once weekly until the high-fat diet group reached 40–45 g (which occurred approximately 16–20 weeks after initiating the high-fat diet). During viral challenge, the average body weight of the lean group was 25 g. Detailed experimental methods, which we previously described elsewhere [18–21], are summarized in the Supplementary Materials.

**Virus Challenge and Therapeutic Intervention With Anti-Leptin Antibody**
Mice were placed under anesthesia and inoculated intranasally with 10^5 median tissue culture infective doses (TCID_{50}) of A[H1N1]pdm09 or 10^3 TCID_{50} of pdmH1N1-Mut in 20 µL of phosphate-buffered saline. Symptoms, body weight, and survival were observed daily for 21 days. Blood and lung samples were collected at predetermined time points [19, 21]. For the therapeutic intervention, 20 µg of goat anti-mouse leptin polyclonal antibody (R&D Systems, Minneapolis, MN) or 20 µg of normal goat immunoglobulin G (IgG) were intravenously injected in groups of 5–7 obese mice through the tail vein immediately before virus challenge.

**Histopathological, Immunostaining, and Viral Load Analysis of Mouse Lung Tissue**
Left-side lungs sampled from each time point were fixed in 10% formalin and processed for histological study. Tissue sections were stained with hematoxylin and eosin for histological examination [22]. Immunohistochemical staining was used for the detection of influenza A virus nucleoprotein (NP) in lung tissue, while immunofluorescent staining was used for dual labeling of influenza A virus NP and the type II pneumocyte marker surfactant protein C (SP-C) to determine the type of cells in mouse lung alveoli that were infected with virus [18, 19]. Leptin receptor was detected by immunofluorescent staining and Western blot, using anti-mouse leptin receptor antibody. Viral load in the right-side lungs were determined by plaque assay [19, 21].

**Detection of Proinflammatory Cytokines, Chemokines, Adipokines, and Prostaglandin E2 (PGE2) in Lung Homogenates**
Levels of proinflammatory cytokines, chemokines, and PGE2 in the lung homogenates and sera were determined by enzyme immunoassay (R&D Systems) [18, 19, 21].

**Detection of Influenza Virus NP–Positive Cells in a Single-Lung-Cell Suspension From Mice**
Influenza virus NP–positive cells from a single-lung-cell suspension prepared from infected mice on day 4 after infection were detected by flow cytometry [11, 19]. Samples stained only with secondary antibody were used to set the gate for NP- or SP-C–positive staining. NP- or SP-C–positive cells on a gated total lung cell population were analyzed.

**Detection of Interleukin 6 (IL-6) Expression in Mouse Lung Cell and Macrophage Cell Lines Stimulated With Recombinant Mouse Leptin**
IL-6 expression in mouse lung cell and macrophage cell lines was measured by quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR) performed as described previously [23]. The expression of β-actin was quantified from all samples by qRT-PCR and used for RNA normalization. A ΔΔCt method was used to estimate the differential gene expression between samples. Fold-changes in each samples were compared with values for an untreated cell control. Mouse single-lung-cell suspension was prepared as described previously [24, 25].

**Statistical Analysis**
Mouse survival rates after infection were analyzed by the Kaplan-Meier method and log-rank test, using SPSS 17.0 for Windows (SPSS, Chicago, IL). Pulmonary viral load and cytokine and chemokine profiles were analyzed by the Student t test. P < .05 was considered statistically significant.
RESULTS

A(H1N1)pdm09 Caused High Mortality in Mice With Diet-Induced Obesity

To study the severity of diseases caused by A(H1N1)pdm09 infection in mice with diet-induced obesity, A(H1N1)pdm09 and pdmH1N1-mut were used. Intranasal inoculation of $10^5$ TCID$_{50}$ of A(H1N1)pdm09 caused death in 66.6% of obese mice and 33.3% of age-matched lean mice (Figure 1A). The mean time to death was also shorter in obese mice, compared with lean mice (9 days vs 12 days). Higher mortality (70% vs 30%) and shorter mean time to death (7 days vs 9 days) were also observed in obese mice infected with only $10^3$ TCID$_{50}$ pdmH1N1-mut when compared to control lean mice (Figure 1B). For all subsequent experiments, pdmH1N1-mut was used.

Histopathological Changes in the Lung

Immunohistochemical staining of influenza virus NP revealed differences in the locations of virus replication in the lungs of obese and lean mice. For obese mice, NP-positive pneumocytes were distributed more peripherally in the pulmonary alveoli on day 2 and day 4 after infection. For lean mice, virus-infected cells were mainly bronchial and bronchiole epithelial cells, with only sporadic NP-positive pneumocytes located more centrally around the infected bronchus (Figure 2Aa and 2Ab). NP-positive pneumocytes were mostly double positive for SP-C, indicating that these cells were mainly type II pneumocytes (Figure 2Ac, 2Ad, and 2Ae). To verify this finding quantitatively, lungs from 3 mice in each infected group were collected on day 4 after infection for preparation of a single-lung-cell suspension by enzymatic digestion, stained with anti-NP and anti-SP-C antibody, and detected by flow cytometry. The results showed that obese mice had a significantly higher percentage of NP-positive lung cells than lean mice. Furthermore, the percentages of cells positive for both NP and SP-C were significantly higher in the lungs of obese mice, compared with lean mice (Figure 2Af). However, these differences were no longer observed after post-infection day 6 (data not shown). These findings suggested that virus infection spread more peripherally to the pulmonary alveoli of obese mice after intranasal challenge.

There was no difference in histological findings between uninfected obese and lean mice (data not shown). Histological examination of the lungs revealed different types of pathological changes at the early stage of infection. Besides vascular congestion, which was seen in both obese and lean mice, lungs of obese mice showed diffuse alveolar wall edema and alveolar space exudation on day 2 after infection (Figure 2B). Alveolar cell necrosis became evident by day 4 after infection, while in control lean mice perivascular lymphocytes and monocytes or peribronchial infiltration were the main histological changes on day 2, and some degrees of bronchial epithelial cell necrosis were observed on day 4 (Figure 2B). After day 4, these pneumonic changes progressed in both obese mice and lean mice but were more severe in obese mice.

Viral Load and Pulmonary and Serum Cytokine/Chemokine Profiles

For the comparison of virus replication kinetics in mouse lungs, viral titers in lung homogenates were determined by plaque assay. The pulmonary viral titer peaked on day 4 after infection in obese mice, 2 days earlier than for lean mice (Figure 3A). The viral load for obese mice on day 4 was significantly higher than that for lean mice ($P < .001$). By day 6 or day 9, no significant difference in pulmonary viral titer was observed between obese and lean mice.

Proinflammatory cytokine and chemokine profiles in lung homogenates showed that, compared with lean mice, those of obese mice exhibited a significantly higher levels of IL-6, interleukin 1β (IL-1β), macrophage inflammatory protein 1α (MIP-1α), and macrophage inflammatory protein 2 (MIP-2).
Figure 2. Histopathological changes of the lung in obese and lean mice. A, Deparaffinized and rehydrated lung tissue sections were incubated with mouse anti-influenza nucleoprotein (NP) protein antibody, followed by biotin-conjugated goat anti-mouse immunoglobulin G secondary antibody (a, b; original magnification ×200). Viral nucleoprotein (NP) was labeled brown by 3, 3′-diaminobenzidine. Representative images of lungs on day 2 after Leptin and Influenza.
on day 4 after infection. On day 6, the level of IL-1β in the lungs of obese mice was still significantly higher than that for lean mice (P = .042; Figure 3B), while no significant difference was observed in levels of IL-6, MIP-1α, and MIP-2 between the 2 groups. The pulmonary level of PGE2 was higher in obese mice, compared with lean mice (Supplementary Figure 1). Serum levels of IL-6 and IL-1β were significantly higher on day 4 in obese mice, compared with lean mice (Figure 3C).

Levels of Circulating Adiponectin and Leptin
Next, we compared the adipokine levels between obese and lean mice. Because there is a close correlation between blood adipokine levels and weight changes [26, 27], we analyzed the adipokine levels after adjustment for body weight (Figure 4). The unadjusted serum adipokine levels are shown in Supplementary Figure 2. Obese mice had a significantly higher level of serum leptin, compared with lean mice, before and after virus infection (P < .01 at all time points tested; Figure 4A). In obese mice, the serum leptin level varied before and after infection, but the changes were not statistically significant. Unlike the leptin level, the serum adiponectin level in obese mice was significantly lower than that in lean mice before viral infection (P = .004) and remained at a lower level (Figure 4B), with no significant changes after viral infection throughout the study. As for lean mice, no significant serial change in serum adiponectin level was observed, compared with the level before infection, except on postinfection day 6 (P = .035; Figure 4B).

Distribution of Leptin Receptor in the Lung
Leptin receptor expression by immunofluorescent staining of lung tissue was found in the epithelial cells of bronchioles and pneumocytes (Figure 5Aa and 5Ab). No visible difference in the distribution and amount of leptin receptor was found in the lung tissue between the obese and lean mice (Figure 5B).

In Vitro Stimulation of Mouse Lung Cells and Cell Lines by Recombinant Mouse Leptin
To determine the effect of leptin on the level of proinflammatory cytokine expression in lung cells and macrophages, recombinant leptin was added to mouse single-lung-cell preparations, a mouse macrophage cell line, and a mouse lung epithelial cell line. Recombinant mouse leptin upregulated the expression of IL-6 messenger RNA in a A(H1N1)pdm09-infected single-lung-cell population (Figure 6A), in mouse macrophage cell line J774A.1 (Figure 6B), and in mouse lung epithelial cell line LA-4 (Figure 6C).

Anti-Leptin Antibody Treatment of Infected Obese Mice
To further explore the role of leptin in severe influenza among obese mice, we used anti-leptin antibody to neutralize the effect of circulating leptin in obese mice infected with pdmH1N1-mut. A single intravenous injection of anti-leptin antibody significantly improved the survival rate to 80%, compared with 40% in the control group treated with normal IgG (Figure 7A). This improvement in survival was associated with a significant reduction in pulmonary levels of the proinflammatory cytokines IL-6 and IL-1β in anti-leptin antibody–treated mice (Figure 7B), but MIP-1α and MIP-2 in the lungs were not affected by this treatment (Figure 7C). However, there was no difference in the pulmonary viral titer on day 4 after infection between the anti-leptin antibody treatment group and the untreated group (Figure 7D). Collectively, the results showed that anti-leptin neutralizing antibody improved survival, with reduction of pulmonary inflammation but not viral replication.

DISCUSSION

Obesity was an independent risk factor for severe influenza during the 2009 pandemic [5]. Many postulations have been put forth to explain how obesity is a predisposing factor for severe influenza [17, 28, 29], but the exact mediator linking obesity and severe influenza has not been established. In this study, we explored the role of adipokines as the mediator for severe influenza, since both leptin and adiponectin are known to regulate the inflammatory response. We first confirmed that mice with diet-induced obesity had a higher mortality rate than control lean mice, and we also detected a higher initial pulmonary viral titer, more extensive and peripheral parenchymal involvement, and more severe lung inflammation in obese mice, compared with lean mice. Next, we determined the role of leptin in severe influenza in obese mice. Body weight–adjusted serum leptin levels were higher in obese mice than in lean mice. In mouse single-lung-cell preparations, mouse lung epithelial cell lines, and mouse macrophage cell...
Figure 3. Viral burden and cytokine/chemokine profile in infected obese and lean mice. A, Viral titer in mouse lung homogenate on days 2, 4, 6, and 9 after infection. Obese and lean mice were infected with 10^3 median tissue culture infective doses of mouse-adapted mutant 2009 pandemic influenza A virus subtype H1N1. Five to 8 mice from each group were euthanized, and right-side lungs were homogenized in 1 mL of minimum essential medium (MEM). Pulmonary viral loads were detected by plaque assay on a Madin-Darby canine kidney cell monolayer. Error bars indicate SDs. B and C, Proinflammatory cytokines and chemokines in mouse lung homogenate (B) and serum (C) were detected by enzyme immunoassay. On days 2, 4, and 6 after infection, the right-side lungs from infected mice (5–8 mice from each group) were homogenized in 1 mL of MEM, and homogenates were used for detection after being clarified by centrifugation. Blood from 5 mice of each group was collected at predetermined times. Sera were separated and used for cytokine detection. Lung and serum specimens from uninfected mice were used as baseline controls. Error bars indicate SDs. Abbreviations: IL-1β, interleukin 1β; IL-6, interleukin 6; MIP-1α, macrophage inflammatory protein 1α; MIP-2, macrophage inflammatory protein 2; PFU, plaque-forming units.
lines, recombinant leptin induced a high level of IL-6 expression. Most importantly, intravenous anti-leptin antibody improved the survival of infected obese mice. This improved survival was associated with a marked reduction in the expression of the proinflammatory cytokines IL-6 and IL-1β but not in pulmonary viral titers, suggesting that the proinflammatory

![Figure 4](image-url) **Figure 4.** Body weight–adjusted serum adipokine levels in infected and uninfected obese and lean mice. Eight to 10 mice from each infected group were euthanized at indicated times after infection. Serum concentrations of leptin (A) and adiponectin (B) were determined by enzyme immunoassay. The mean leptin and adiponectin concentrations were divided by the mean body weight. Error bars indicate SDs. *P < .05 for the comparison between infected and uninfected lean mice.

![Figure 5](image-url) **Figure 5.** Expression of leptin receptor in mouse lung tissue as determined by immunofluorescent staining and Western blot. A, Infected obese mice (a and c) and lean mice (b and d) on days 2, 4, and 6 after infection were euthanized, and left-side lungs from 3 mice in each group were fixed in formalin and sectioned after embedding in paraffin. Tissue sections were immunostained with goat anti-mouse leptin receptor antibody, followed by staining with Texas red–conjugated donkey anti-goat immunoglobulin G (IgG). Representative images are from an infected mouse lung showing pneumocytes (a and b) and bronchiolar epithelial cell membrane (c and d) positive for leptin receptor (original magnification ×400). B, Total protein was extracted from uninfected or infected mouse lungs. Equal amounts of protein were separated by 8% sodium dodecyl sulfate polyacrylamide gel electrophoresis and blotted for detection by rabbit anti-mouse leptin receptor antibody and secondary antibody of goat anti-rabbit IgG-HRP. Two bands with molecular weights of 125 kD and 100 kD were detected; protein extracts from mouse macrophage cell line RAW264.7 were used as a positive control.
effect of leptin could partly be mediated via IL-6 and IL-1β. Thus, we have established the role of leptin in the pathogenesis of severe influenza, and we have identified a potential host target for the treatment of influenza in obese patients. The serum adiponectin level was also lower in obese mice, compared with lean mice. However, the magnitude of the difference in adiponectin levels between obese and lean mice was much lower than that for leptin.

At the early stage of A(H1N1)pdm09 infection, obese mice were less effective in controlling viral replication, as indicated by the more extensive spread of the virus to the periphery of the lung. The abundant number of type II pneumocytes infected with A(H1N1)pdm09 at the early stage of infection in lungs from obese mice may have contributed to the alveolar cell necrosis and inflammatory alveolar exudation. Diffuse alveolar edema observed at the early stage was also associated with a high level of PGE2 in lungs from obese mice. We found a very high level of PGE2 in lung homogenate for obese mice, compared with lean mice. PGE2 is considered a potent proinflammatory mediator that causes major vascular changes of vascular dilatation and increased permeability at the sites of infection [30, 31].

Leptin has been considered to be a proinflammatory adipokine that is associated with the chronic systemic inflammation in obese individuals. In addition to adipose tissue, leptin is also secreted by bronchial epithelial cells, type II pneumocytes, and lung macrophages, and it acts in the airways via leptin receptors [32]. A higher leptin level in bronchoalveolar fluid has been associated with higher mortality in patients with acute respiratory distress syndrome [33]. Some previous studies also suggested that a higher leptin level is associated with more severe asthma, chronic obstructive pulmonary disease, and obstructive sleep apnea syndrome, although the data were conflicting [34, 35]. In the current study, we found a significantly higher level of serum leptin throughout the observational period, before and after infection in obese mice, which is similar to the results from a study using the influenza A virus PR8 strain [12]. Although substantial reductions in the serum leptin level were observed during illness for infected obese mice, the decreases were no longer statistically significant after adjustment for the loss of body weight after infection. In the study by Smith et al, the leptin level in obese mice decreased and then rebounded on day 6 after infection [12]. This difference may be related to the change in body weight observed during virus infection, which was not included in their analysis. Leptin is mainly produced by adipocytes, and the serum leptin level is proportional to body weight [26]. In our study, an average reduction of 10% in the body weight of obese mice after infection (data not shown) was consistent with the observation by O’Brien et al [16]. Therefore, the changes in serum leptin level in their obese mice during virus infection were mainly related to changes in body weight. Nevertheless, the preexisting elevated leptin level in obese mice before infection may sensitize the lung macrophages and other types of cells in the respiratory system to a proinflammatory mode. Once A(H1N1)pdm09 infection was established, these

Figure 6. Quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR) detected changes in the interleukin 6 (IL-6) messenger RNA (mRNA) level in a mouse single-lung-cell population (A), mouse macrophage cell line J774A.1 (B), and mouse lung epithelial cell line LA-4 (C). Cells or cell lines were cultured for 24 hours before infection with mouse-adapted mutant 2009 pandemic influenza A virus subtype H1N1 at a multiplicity of infection of 5. Serial concentrations of recombinant mouse leptin protein were added and further cultured for 24 hours. The cells were harvested for RNA extraction, reverse transcription, and qRT-PCR detection of IL-6 mRNA. β-actin mRNA was used for normalization of the RNA concentration. A mouse single-lung-cell suspension was prepared from 3 uninfected obese mice and 3 nonobese control mice by enzymatic digestion of the lung. The obtained whole lung cell population in the single-cell suspension was cultured for 24 hours in Roswell Park Memorial Institute 1640 medium supplemented with 10% fetal bovine serum, 1% penicillin/streptomycin, and 50 μM β-mercaptoethanol.
cells produced large amounts of proinflammatory cytokines, contributing to the severe inflammatory changes observed in obese mice lungs. In further studies, it would be interesting to find out the functional activity of the receptors and whether the tyrosine phosphorylation status of the receptors changed in obese versus lean mice.

By using anti-leptin antibody to antagonize the effect of leptin in vivo, we improved survival, with marked reduction of pulmonary inflammatory response despite little change in pulmonary viral titer. This finding suggested that leptin has no effect on viral replication and corroborated with the findings of in vitro stimulation in a mouse single-lung-cell population. When the macrophages and lung epithelial cells were stimulated with recombinant mouse leptin, increased transcription of proinflammatory cytokines was evident in infected cells, compared with unstimulated controls. Our results from mice with diet-induced obesity were different from those of ob/ob mutant obese mice with leptin deficiency. Studies involving ob/ob mice showed that an abnormally low level of leptin predisposed these mice to more severe bacterial pneumonia [36] and that exogenous leptin improved survival [37]. A functional leptin pathway has been shown to be important in pulmonary host defense [38, 39]. Therefore, both abnormally high or low levels of leptin are associated with an unfavorable immune response. Since most obese patients have high serum leptin levels, anti-leptin antibody may represent a new form of immunomodulatory therapy in addition to other reported strategies [40]. The exact mechanism of anti-leptin antibody in antagonizing the effect of leptin should be further investigated in the future.

In contrast to leptin, adiponectin has been considered to be an antiinflammatory adipokine, and lower levels of adiponectin have been observed in patients with sepsis [41]. Proinflammatory cytokines such as tumor necrosis factor α suppress adiponectin production in adipocytes, and adiponectin has also been shown to induce the production of antiinflammatory cytokines, such as IL-10 and IL-1 receptor antagonist [42]. Thus, a low serum adiponectin level constitutes a lack of suppressor control, which may contribute to the proinflammatory state in mice with diet-induced obesity that are infected with A(H1N1)pdm09 [28]. Acute lung injury is also more severe in adiponectin-deficient mice [43]. In the current study, body weight–adjusted serum adiponectin concentrations were...
significantly lower in obese mice than in lean mice before and after viral infection. Whether this low serum adiponectin level contributed to the severe inflammation caused by pdmH1N1-mut infection in obese mice warrants further investigation. There were no significant serial changes in body weight–adjusted adiponectin levels in lean mice, except on day 6 after infection, but there was significant reduction in adiponectin levels without body weight adjustment on both day 4 and day 6 after infection. This reduction in the adiponectin level may be related to the fasting state during acute illness [44].

There are several limitations in this study. First, we used a diet-induced mouse model with a high level of leptin, which differs from genetically predisposed obese mice deficient in serum leptin. The degree of leptin resistance also differs between these 2 types of mice [45]. Consequently, the results from this study may not be applicable to genetically obese mice. Second, the exact pathway linking leptin and pulmonary inflammation in vivo is still unclear. Previous studies demonstrated that leptin activates the intracellular signaling Janus kinase 2 (JAK2)–signal transducer and activation of transcription factor 3 (STAT3) pathway in cultured murine macrophages [46, 47] and human monocytes [48] and induces JAK2-STAT3 and mitogen-activated protein kinase in mouse hypothalamus [49]. Further studies are required to delineate the exact pathways. Third, a high leptin level in obese animals is often associated with leptin refractoriness. This possibility should be explored in this mouse model, especially in relation to anti-leptin antibody treatment, in future studies.

Many obese patients with severe influenza died during the 2009 pandemic despite intensive care admission and antiviral treatment. Understanding the mechanism leading to severe disease in these individuals is the cornerstone for developing effective adjunctive therapy in addition to the specific antiviral therapy. In this study, we have demonstrated that a dysregulated inflammatory response with a suboptimal early viral control and severe inflammatory damage occurred in lungs of infected obese mice. A high leptin level was associated with more severe inflammatory damage in infected obese mice, and anti-leptin antibody was effective in reducing mortality without decreasing the pulmonary viral load in our diet-induced mouse model. Our findings suggest that modulating the proinflammatory response is necessary to improve the survival of obese patients, especially because they often present >48 hours after symptom onset, when the window of opportunity for useful antiviral treatment has passed [9, 50]. Studies to determine safe ways to downmodulate serum leptin levels in obese subjects with severe influenza are warranted.

**Supplementary Data**

Supplementary materials are available at The Journal of Infectious Diseases online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

### Notes

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