Impaired Wound Healing Predisposes Obese Mice to Severe Influenza Virus Infection

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(See the editorial commentary by Beck, on pages 172–3, and the article by Kim et al, on pages 244–51.)

For the first time, obesity appeared as a risk factor for developing severe 2009 pandemic influenza infection. Given the increase in obesity, there is a need to understand the mechanisms underlying poor outcomes in this population. In these studies, we examined the severity of pandemic influenza virus in obese mice and evaluated antiviral effectiveness. We found that genetically and diet-induced obese mice challenged with either 2009 influenza A virus subtype H1N1 or 1968 subtype H3N2 strains were more likely to have increased mortality and lung pathology associated with impaired wound repair and subsequent pulmonary edema. Antiviral treatment with oseltamivir enhanced survival of obese mice. Overall, these studies demonstrate that impaired wound lung repair in the lungs of obese animals may result in severe influenza virus infection. Alternative approaches to prevention and control of influenza may be needed in the setting of obesity.

In March 2009, a novel influenza A virus subtype H1N1 strain derived from 2 preexisting swine influenza virus lineages was identified in Mexico and the United States [1]. By June 2009, the World Health Organization indicated the start of the first influenza pandemic of the 21st century. The actual number of cases worldwide is unknown as most cases were not laboratory confirmed. However, estimates suggest that the total number of cases was on the order of several tens of millions worldwide [2–4]. Although most illness associated with infection was mild and self-limiting [4], several high-risk groups, including persons with chronic illnesses, pregnant women, and immunocompromised patients, experienced more severe infection [4–6]. Strikingly, however, obesity was also shown to be a risk factor for developing severe influenza disease [3, 5–8]. Severe or morbid obesity was associated with a relative risk of severe disease or death between 5 and 15 times greater than that of the rest of the population [9].

Obesity is increasing epidemically worldwide [10, 11]. Although the role of obesity as a risk factor for increased morbidity and mortality through its association with cardiovascular disease and diabetes is well documented [12–14], the past decade has highlighted that obesity is also a risk factor for respiratory diseases (reviewed in [15]). The exact mechanisms are unclear; however, obesity is associated with a state of chronic systemic inflammation that may promote airway hyperresponsiveness [15]. A recent study describing the clinicopathologic characteristics of confirmed pandemic 2009 H1N1-associated deaths found that 72% of the cases were in obese adults and adolescents [8]. Others, including groups in Chile, Canada, and Mexico, reported that obesity was one of the most frequently identified underlying conditions in fatal pandemic 2009 H1N1 influenza cases in patients over 20 years of age [16]. Given the worldwide increase in obesity, there is a pressing public health need to understand the pathogenic mechanisms underlying poor outcomes from influenza infection in this expanding...
population. The goal of the present study was to fill this gap in knowledge by examining the severity of pandemic 2009 H1N1 virus in obese animals and evaluate antiviral therapy effectiveness with the neuraminidase (NA) inhibitor oseltamivir. Our data confirm that obese animals are more susceptible to severe influenza virus infection. Increased pathogenicity appears to be due to impaired wound repair in the infected lungs of obese animals, leading to edema rather than increased replication or spread of the virus. Importantly, oseltamivir protected obese animals. Overall, these studies demonstrate that the enhanced inflammation and lack of wound repair in obese mice may lead to the development of more severe influenza infection but that antiviral therapies are effective in this population.

METHODS

Ethics Statement
All procedures involving animals were approved by the St. Jude Children’s Research Hospital Institutional Animal Care and Use Committee and were in compliance with the Guide for the Care and Use of Laboratory Animals.

Animals

Genetically Obese Mice
Eight-week-old lean male C57BL/6J (~21 g) and B6.V-Lepob/J (genetically obese [ob/ob]; ~53 g) mice were obtained from Jackson Laboratory (Bar Harbor, ME).

Diet-Induced Obese Mice
Eleven-week-old male C57BL/6J (~21 g) and preconditioned diet-induced obese (DIO; ~35 g) mice were obtained from Harlan Laboratories (Indianapolis, IA). The mice were preconditioned by feeding C57BL/6 mice either a traditional mouse diet or a Tekland high-fat diet (60% Kcal from fat; TD.06414, Tekland, Harlan Laboratories) from the time of weaning. All mice had free access to food and water.

Viruses and Infection
A/California/04/2009 (CA/09, pandemic H1N1 [pH1N1]) and A/Hong Kong/1/1968 2:6 (HK68, influenza A virus subtype H3N2 [17]) viruses were propagated in the allantoic cavity of 10-day-old specific pathogen-free embryonated chicken eggs. At 48–72 hours postinfection, allantoic fluid was harvested, clarified by centrifugation, and stored at −70°C. Viral titers were determined by 50% tissue culture infectious dose (TCID50) analysis in Madin-Darby canine kidney (MDCK) cells as described elsewhere [18] and evaluated by the method of Reed and Muench [19]. For infections, mice were lightly anesthetized by isofluorane inhalation and intranasally inoculated with 10^5 TCID50 of CA/09 in 25 μL phosphate-buffered saline (PBS) (0.1 median lethal dose [MLD50]) or 6.3 × 10^5 TCID50 of HK68 in 100 μL PBS (0.2 MLD50).

Flow Cytometry
Bronchoalveolar lavage (BAL) samples were collected on days 3–14 postinfection from 2 control and 3 infected mice, and flow cytometry was performed. Briefly, single-cell populations were collected by mild centrifugation (100g, 10 minutes), resuspended in red blood cell lysis solution (0.15 mol/L NH4Cl, 1 mmol/L KHCO3, and 0.1 mmol/L ethylenediaminetetraacetic acid), washed with PBS, and aliquoted at 1.0 × 10^6 cells per sample. Aliquots then were incubated with 4% normal rat serum and anti-Fc block (CD16/32; eBioscience Inc, San Diego, CA) for 30 minutes at 4°C. Cell populations were stained with Ly-6G, CD11b, CD11c, F4/80, CD86, NK1.1, CD3, CD4, CD8, and CD69 antibodies directly conjugated to Alexa Fluor 647, Alexa Fluor 700, fluorescein isothiocyanate, phycoerythrin, and APC-eFluor (eBioscience Inc) combined with the violet LIVE/DEAD Fixable Dead Cell Stain Kit (Invitrogen, Carlsbad, CA). The BD LSRII flow cytometer (BD Biosciences) was used for cellular acquisition of 10 000 total live, singlet events per sample, and results were analyzed using FlowJo Flow Cytometry Analysis software.

Quantitation of Viral Titers
On days 3–14 postinfection, lungs were removed from 2 control and 3 infected mice per group, homogenized in minimum essential medium, and stored at −80°C. Titers determined on individual mice were performed in triplicate by TCID50 analysis in MDCK cells [18] and evaluated by the method of Reed and Muench [19].

Luminex Cytokine Arrays
Briefly, lung homogenates were collected and cytokine/chemokine levels determined using the Milliplex Mouse 22-Plex Cytokine Detection System (LINCO Research Inc, Saint Charles, MO) on a Luminex100 reader (Luminex Corp, Austin, TX). Data were calculated using a calibration curve obtained in each experiment using the respective recombinant proteins as per manufacturer’s instructions. Values were normalized to equivalent protein concentrations as determined by BCA Protein Assay (Pierce, IL) and calculated as the mean of replicates of 6.

Histopathology
On days 3–14 postinfection, deeply anesthetized mice were perfused with 4% paraformaldehyde. Immunohistochemical staining was performed by the St. Jude Children’s Research Hospital Veterinary Pathology Core Facility.

Oseltamivir Treatment
Oseltamivir phosphate (oseltamivir) was administered by oral gavage (100 μL/mouse) twice daily for 5 days to groups (n = 5) at dosages of 20 and 100 mg/kg/day. Control (infected untreated) mice received sterile PBS on the same schedule. Six hours after receiving the first dose of oseltamivir, the mice were inoculated with 10^5 TCID50 of CA/09 in 25 μL PBS [20].
Statistical Analysis
Comparison of survival between groups of mice was performed with the log rank \( \chi^2 \) test on the Kaplan–Meier survival data. Comparison of the cellular, cytokine, and viral titer data was performed using analysis of variance for multiple comparisons or Student \( t \) test for pairwise comparisons with GraphPad Prism (GraphPad, San Diego, CA). Error bars represent standard deviation, and statistical significance was defined as \( P < .05 \).

RESULTS

Survival of Obese Mice Challenged With Pandemic H1N1 and H3N2 Influenza Viruses
Epidemiological studies suggested that obesity was a risk factor for developing severe 2009 H1N1 influenza infection [3, 5–9]. To evaluate the relevance of obesity as a risk factor for developing severe influenza and begin defining underlying mechanisms, strain-matched lean (C57BL/6, \( n = 28 \)), and DIO (\( n = 28 \)) or ob/ob (\( n = 28 \)) mice were lightly anesthetized and intranasally inoculated with CA/09 pH1N1 virus and monitored for morbidity and mortality. Both genetically and diet-induced obese mice were used to more closely mimic causes of human obesity. The pH1N1 virus-infected mice in all groups lost \( \sim 10\%–15\% \) of their starting weight by day 6 postinfection with the lean mice continuing to lose weight at day 8 postinfection (Figure 1A). Despite a lack of significant weight loss, obese mice rapidly succumbed to pH1N1 infection (Figure 1B). Within 6 days postinfection, 80% of the infected obese mice died as compared with \( \sim 20\% \) of the lean controls (\( P < .01 \) for DIO and \( P < .003 \) for ob/ob). The enhanced mortality did not differ with the type of obesity and was not specific to the 2009 pH1N1 influenza virus. Obese mice infected with the 1968 HK68 H3N2 virus also succumbed more readily to infection (Figure 1C). With this virus, 100% of the ob/ob mice died by day 6 postinfection, whereas the DIO had a slight, although significant delay in mortality, with 100% dying by day 8 postinfection (\( P < .004 \)). Similar results were observed with highly pathogenic H5N1 viruses (data not shown). These results suggest that obese mice are more likely to develop severe influenza infection.

Immune Cell Infiltration in Obese Mice
Obesity is associated with chronic low levels of inflammation and airway hyperresponsiveness (reviewed in [15, 21]), so we further examined lung inflammation. Immune cell infiltration into the airways was monitored by BAL at days 3, 6, and 14 postinfection. At day 3 postinfection, infiltrating monocyte (CD11b\(^+\), CD11c\(^+\), Ly-6g\(^-\), and CD86\(^+\)) levels increased in the pH1N1-infected obese mice as compared with lean mice (\( P < .01 \); Figure 2A). The infected ob/ob mice also had elevated levels of neutrophils (CD11b\(^+\), CD11c\(^+\), Ly6g\(^hi\), and F4/80\(^+\)) and CD8\(^+\) \( T \) cells (CD3\(^+\), NK1.1\(^-\), CD8\(^+\), and CD4\(^-\); \( P < .01 \); Figure 2A). By day 6 postinfection, all the cell types monitored were significantly increased in the obese mice with the most significant elevation being in neutrophil numbers (\( P < .001 \); Figure 2B). Neutrophil numbers were \( \sim 9\)-fold higher in the ob/ob and \( \sim 20\)-fold higher in the DIO mice (3.5 \( \times \) 10\(^6\) and 7.2 \( \times \) 10\(^6\), respectively, as compared with 3.8 \( \times \) 10\(^5\) cells in lean mice). The increased cellular infiltration was still evident at day 14 postinfection in the obese mice that survived viral infection (Figure 2C). The elevated cellular infiltration was likely due to amplified levels of the chemokines granulocyte colony-stimulating factor (G-CSF), CXCL10 (IP-10), CXCL1 (KC), and monocyte chemoattractant protein-1 (MCP-1)
present in the lungs of the infected obese mice (Table 1). This was accompanied by elevated levels of the proinflammatory cytokine interleukin 6 (IL-6). We found significant decreases in the levels of the immunoregulatory protein transforming growth factor β at day 3 postinfection and interferon γ at day 6 postinfection as compared with both uninfected control and infected lean mice (Table 1), suggesting further dysregulation of immune responses.

**Histopathology in Lungs of Obese Mice Infected With pH1N1 Influenza Virus**

Histologically, there were no significant differences among the lean, DIO, and ob/ob pH1N1-infected mice on days 2 and 3 postinfection (data not shown), and by day 5 postinfection, all of the mice had increased interstitial inflammation and moderate perivascular cuffing by lymphocytes and neutrophils (Figure 3A). At day 6 postinfection, the overall severity and extent of inflammation in the lungs of mice in all 3 groups increased, although there were differences in the severity. In lean mice, alveoli frequently contained abundant granulocytes and cell debris, and perivascular cells were composed largely of macrophages and granulocytes accompanied by variable numbers of lymphocytes (Figure 3A). The lungs of infected DIO mice had similar lesions but showed a greater extent and severity of granulocytic inflammation and protein exudates in alveoli (Figure 3A). Unexpectedly, the ob/ob mice showed significantly less interstitial and alveolar inflammation, yet there was increased edema (Figure 3A). However, at day 14 postinfection, there was still significant inflammation in the lungs of the obese mice in contrast to the lean mice (Figure 6A), suggesting that the obese animals had delayed resolution of lung inflammation following influenza virus infection. No lesions were seen outside the lung.

To further evaluate the effect of obesity on lung pathology, we next examined epithelial proliferation using the cellular marker for proliferation Ki-67 [22]. A striking finding was the marked reduction in the extent of epithelial regeneration in the infected obese animals as determined by immunohistochemical staining (Figure 3B). At day 6 postinfection, evidence of epithelial regeneration was widespread in the bronchioles of lean mice, whereas the majority of the bronchiolar surfaces in DIO mice remained denuded of epithelial covering. The relatively few bronchioles that showed regeneration in DIO mice were typically only partially covered by attenuated epithelial cells. The reduction in epithelial regeneration was also severe in the ob/ob-infected mice. Although some bronchioles were denuded of epithelium, much of the luminal surface of bronchioles were covered by a discontinuous layer of regenerating epithelium, which in many bronchioles appeared as a flattened attenuated single layer of cells. Quantitative analysis showed a decrease in Ki-67 staining from ~59% positive cells in lean mice to ~43% in DIO mice and 38% in ob/ob mice (P < .005; Figure 3C). The ob/ob infected mice also had significantly less Ki-67 staining than did the DIO mice (P < .05). The decreased Ki-67 staining in the obese mice was still evident at day 14 postinfection, suggesting an impaired wound repair response (Figure 3C).
### Table 1. Pulmonary Expression of Cytokines/Chemokines in Uninfected and pH1N1-Infected Obese Mice

<table>
<thead>
<tr>
<th>Cytokine/Chemokine</th>
<th>Day 3 Infected Lean</th>
<th>Day 3 Uninfected Lean</th>
<th>Day 6 Infected ob/ob</th>
<th>Day 6 Uninfected ob/ob</th>
<th>Day 3 Infected DIO</th>
<th>Day 3 Uninfected DIO</th>
<th>Day 6 Infected ob/ob</th>
<th>Day 6 Uninfected ob/ob</th>
</tr>
</thead>
<tbody>
<tr>
<td>G-CSF</td>
<td>9 ± 2</td>
<td>11 ± 3</td>
<td>1 ± 0.2</td>
<td>60 ± 4</td>
<td>3441 ± 635</td>
<td>2518 ± 401</td>
<td>4 ± 2</td>
<td>1 ± 0.1</td>
</tr>
<tr>
<td>IL-6</td>
<td>0.4 ± 0</td>
<td>1 ± 0.3</td>
<td>745 ± 218</td>
<td>40 ± 1</td>
<td>1074 ± 144</td>
<td>569 ± 198</td>
<td>0.4 ± 0.2</td>
<td>1 ± 0.7</td>
</tr>
<tr>
<td>CXCL10</td>
<td>80 ± 8</td>
<td>32 ± 10</td>
<td>17 ± 9</td>
<td>69 ± 41</td>
<td>607 ± 89</td>
<td>745 ± 150</td>
<td>18 ± 4</td>
<td>18 ± 5</td>
</tr>
<tr>
<td>CXCL1</td>
<td>20 ± 9</td>
<td>25 ± 2</td>
<td>19 ± 6</td>
<td>22 ± 8</td>
<td>102 ± 17</td>
<td>724 ± 153</td>
<td>5 ± 4</td>
<td>225 ± 60</td>
</tr>
<tr>
<td>MCP-1</td>
<td>4 ± 0.3</td>
<td>2 ± 0.2</td>
<td>2 ± 0.5</td>
<td>6 ± 0.04</td>
<td>80 ± 21</td>
<td>58 ± 0.3</td>
<td>1 ± 0.1</td>
<td>1 ± 0.1</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>2348 ± 375</td>
<td>1790 ± 103</td>
<td>1984 ± 390</td>
<td>4237 ± 373</td>
<td>585 ± 277</td>
<td>390 ± 279</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>TGF-β</td>
<td>15 ± 4</td>
<td>171 ± 1</td>
<td>468 ± 1</td>
<td>170 ± 16</td>
<td>34 ± 4</td>
<td>8 ± 0.3</td>
<td></td>
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</tr>
</tbody>
</table>

**Abbreviations:** pH1N1, pandemic influenza A virus subtype H1N1; DIO, diet-induced obese; ob/ob, genetically obese; G-CSF, granulocyte colony-stimulating factor; IL-6, interleukin 6; MCP-1, monocyte chemoattractant protein-1; TGF-β, transforming growth factor β; IFN-γ, interferon γ.

* Mean concentration (pg/mL) ± SD from 6 mice.
assessed. In contrast to the untreated mice (Figure 5A), all mice administered oseltamivir had cleared the virus by day 6 post-infection (data not shown) and there was decreased lung inflammation (Figure 6A) as compared with untreated mice (Figure 3A). This was even more evident at day 14 postinfection where there was still significant inflammation in the lungs of the untreated obese mice in contrast to the lean mice (Figure 6A). Oseltamivir treatment led to lessened inflammation in the obese mice with Ki-67 levels similar to lean mice. These studies suggest that influenza virus infected obese mice have prolonged inflammation and impaired wound repair independent of increased viral titers that can be alleviated by treatment with oseltamivir.

**DISCUSSION**

Obesity has become a worldwide epidemic. It is estimated that >30% of adults in the United States are overweight or obese.
Obesity can lead to a variety of serious conditions, and more recent evidence suggests that it is also associated with impaired immune function and increased susceptibility to a number of different pathogens (reviewed in [21]), including influenza virus [3, 28–30]. Experimental studies in obese animals demonstrated augmented mortality during sepsis [31] and decreased viral myocarditis during coxsackievirus B4 infection [32].

In the present study, we show that both genetically and diet-induced obese animals are more likely to develop severe influenza infection. Our results demonstrated that the increased severity was independent of the viral strain and appeared to be due to impaired wound repair in the lungs of infected obese animals leading to edema, and not to increased lung replication of the virus or spread outside the lungs. Importantly, adjusted oseltamivir treatment based on body weight reduced severity of infection and protected obese animals from death.

Our findings complement previous work using the mouse-adapted laboratory strain A/Puerto Rico/8/34 (H1N1; PR8). These studies demonstrated that 50% of PR8-infected DIO mice succumbed to infection by day 8 postinfection [33]. Similar to our data, they found no difference in viral titers in the obese animals as compared with nonobese mice [33]. In that study, infection in obese animals was associated with depressed cytokine levels, reduced natural killer (NK) cell cytotoxicity, and selective impairment in dendritic cell function [34]. Further, they demonstrated that obesity led to alterations in the T-cell populations that may ultimately be damaging to the host [35, 36]. However, the mechanism for the increased mortality during the primary infection was not defined and protection by antiviral therapies was not addressed.

To understand the mechanism of increased mortality during primary infection, we evaluated lung inflammation and function. Although there were no remarkable difference in histopathology at day 3 postinfection, the obese mice had increased numbers of infiltrating monocytes and decreased numbers of NK cells as compared with nonobese animals. The increase in monocytes could be due to the increased expression of the chemokines G-CSF, CXCL1, CXCL10, and MCP1 in the lungs of obese mice. Strikingly, there were remarkable differences in the
infiltrating cell populations depending on the type of obesity. The infected DIO mice had significantly fewer infiltrating neutrophils and CD4\(^+\) and CD8\(^+\) T cells as compared with either lean or ob/ob mice. The reason for these differences remains under investigation.

In contrast, by day 6 postinfection, the obese animals had significantly higher cellular infiltration in the lung that was not resolved by day 14 postinfection. Histologically, the DIO mice had the greatest extent and severity of granulocytic inflammation and protein exudates in alveoli as compared with lean mice. Although the ob/ob mice superficially appeared to have the least severe pulmonary lesions, with notably less interstitial and alveolar cellular infiltrates, mice in this group had the most extensive and severe pulmonary edema. Both obese groups had higher levels of protein and albumin in the lungs as compared with lean mice, suggesting a defect in barrier permeability. Exploring a potential mechanism, we found a notable reduction in the airway reepithelialization in the lungs of obese animals. The reduced extent of wound repair was most evident in the genetically obese animals, suggesting that either the increased weight of the animals or some underlying metabolic complications led to decreased wound healing. Delayed or impaired wound repair is a common finding in obesity. In humans, obesity leads to impaired cutaneous wound repair [37], and more extensive studies in obese animals demonstrated both impaired cutaneous [38] and gastric healing [39]. Studies are underway to determine the cause of the delayed wound repair and its role in disease severity.

Figure 6. Decreased lung histology and increased Ki-67 staining in the lungs of influenza virus–infected mice treated with oseltamivir. A, At days 6 and 14 postinfection, lungs were collected from deeply anesthetized and formalin-perfused mice and paraffin-embedded. Sections (4 \(\mu\)m thick) were stained with hematoxylin and eosin, and representative pictures of each group are shown at 20\(\times\) magnification. B, Digital images of the Ki-67 slides were obtained, and the percentage of positive nuclei in 4 random sections of the lung for each animal were determined with ImageScope using a nuclear-based algorithm. Error bars represent SD. Abbreviations: dpi, days postinfection; Olestam, oseltamivir; DIO, diet-induced obese; ob/ob, genetically obese.
An important question that remained unaddressed was how obesity-related pharmacokinetic and pharmacodynamic changes might affect the efficacy of therapeutic interventions in patients suffering from influenza virus [40, 41]. Thus, we treated obese animals with increasing doses of oseltamivir and monitored protection. Our studies showed that dosing at 20 mg/kg/day increased survival from 40% to 80% and that dosing 100 mg/kg/day afforded complete protection to obese animals. These findings suggest that oseltamivir should be effective in this highly susceptible population and are supported by several reports showing that oseltamivir is the drug of choice for treating severe pH1N1 infections, including those in obese patients [42–44].

In summary, we demonstrate that obesity is a risk factor for severe influenza infection in mice. Furthermore, we implicate poor lung wound healing leading to pulmonary edema suggestive of acute respiratory distress syndrome as a potential underlying mechanism, rather than a lack of viral control. This has implications for the prevention and treatment of influenza in obese persons. Obesity should be recognized as a risk factor requiring early antiviral therapy, and the pharmacokinetics and effectiveness of anti-influenza drugs should be carefully examined in this population. Clearly, further epidemiologic investigation of outcomes during seasonal influenza are indicated, and preparation for the next pandemic should take obesity into account.

Notes

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Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

infection with the 2009 pandemic H1N1 influenza virus. Influenza Other Respi Viruses 2011; 5:418–425.


