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Diet-Induced Obesity Impairs the T Cell Memory Response to Influenza Virus Infection

Erik A. Karlsson, Patricia A. Sheridan, and Melinda A. Beck

The Centers for Disease Control and Prevention has suggested that obesity may be an independent risk factor for increased severity of illness from the H1N1 pandemic strain. Memory T cells generated during primary influenza infection target internal proteins common among influenza viruses, making them effective against encounters with heterologous strains. In male, diet-induced obese C57BL/6 mice, a secondary H1N1 influenza challenge following a primary H3N2 infection led to a 25% mortality rate (with no loss of lean controls), 25% increase in lung pathology, failure to regain weight, and 10- to 100-fold higher lung viral titers. Furthermore, mRNA expression for IFN-γ was >60% less in lungs of obese mice, along with one third the number of influenza-specific CD8$^+$ T cells producing IFN-γ postsecondary infection versus lean controls. Memory CD8$^+$ T cells from obese mice had a >50% reduction in IFN-γ production when stimulated with influenza-pulsed dendritic cells from lean mice. Thus, the function of influenza-specific memory T cells is significantly reduced and ineffective in lungs of obese mice. The reality of a worldwide obesity epidemic combined with yearly influenza outbreaks and the current pandemic makes it imperative to understand how influenza virus infection behaves differently in an obese host. Moreover, impairment of memory responses has significant implications for vaccine efficacy in an obese population.   *The Journal of Immunology*, 2010, 184: 3127–3133.

O besity has become a worldwide epidemic. The World Health Organization predicts that by the year 2015, 2.3 billion adults will be overweight (body mass index [BMI] ≥ 25) with 700 million being classified as clinically obese (BMI ≥ 30) (1). Obesity has been linked to numerous health problems and chronic diseases, including type 2 diabetes, hypertension, dyslipidemia, certain cancers, and cardiovascular diseases (2). These comorbidities have been associated with hormonal and metabolic changes related to an increase in adipose tissue mass (3). Obesity has been well established as a risk factor for increased morbidity and mortality; however, its effects on susceptibility to infection are just beginning to be understood. In the hospital setting, obese patients are more likely to have secondary infections and complications develop, such as sepsis, pneumonia, bacteremia, and wound and catheter-related infections. Patients with increased BMI and adiposity also present a higher incidence of surgical site infections, which have been associated with increased risk of other wound complications, increased length of stay, and increased risk of death (4–8). Obesity also negatively affects pulmonary function and BMI has been correlated to increased susceptibility to community-related respiratory tract infections (9–12).

Influenza is a seasonal respiratory illness caused by the influenza virus. Spread through airborne transmission, these viruses are highly contagious and are responsible for a great deal of morbidity and mortality in the world (13). In any given year, 5–15% of the world population is infected with influenza virus resulting in 3–5 million cases of severe illness and 500,000 deaths from influenza and influenza-related complications (14). Young children, the elderly, and people with chronic diseases are particularly susceptible to influenza-related complications and mortality (15). Influenza viruses undergo continual mutation (drift) in surface Ags and occasional gene reassortment (shift) resulting in heterologous strains with serologically distinct surface proteins. Therefore, a vaccine that promotes a robust memory B cell Ab-based response to the surface proteins of one strain of influenza could be ineffective for a strain encountered the next season expressing different surface proteins (16). By comparison, memory T cells generated during primary influenza infection can target internal proteins common and less variable among influenza strains, making them more effective against encounters with heterologous virus stains (17).

Very recently, the Centers for Disease Control and Prevention has suggested that obese individuals are at a greater risk of morbidity and mortality from pandemic novel influenza H1N1 strain. Speculation on the cause ranges from excess adipose tissue constricting lung volume to chronic inflammation influencing the immune response (18). We hypothesize this increased severity may be due to obesity decreasing memory cell-mediated defenses against heterosubtypic influenza infection. Previously, we demonstrated that influenza infected diet-induced obese (DIO) mice developed increased lung pathology, decreased expression of IFN-α/β and delayed expression of proinflammatory cytokines and chemokines (19). In addition, dendritic cells (DCs) from obese mice failed to efficiently present influenza Ag to T cells (20). Because development and subsequent functionality of T cell-mediated immune memory depends on the primary response (21), we hypothesized that obese mice would have an impaired memory T cell response to a secondary influenza infection.

Materials and Methods

Animals

Weanling, male C57BL/6J mice were obtained from The Jackson Laboratory (Bar Harbor, ME). All mice were housed at the University of North Carolina Animal Facility, which is fully accredited by the American

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Abbreviations used in this paper: BMI, body mass index; DC, dendritic cell; DIO, diet-induced obese; p.i., postinfection; PR8, influenza A/Puerto Rico/8/34; TCID, tissue culture infectious dose.

Association for Accreditation of Laboratory Animal Care. Animals were housed four per cage under pathogen-free/viral Ab-free conditions and maintained under protocols approved by the Institutional Animal Use and Care Committee. Mice were randomized to receive either a low-fat/no-sucrose diet or a high-fat/high-sucrose diet for 20 wk. The diets, previously described by Surwit et al. (22, 23), were obtained from Research Diets (New Brunswick, NJ). The low-fat diet (D12328) consisted of 16.4% protein, 73.1% carbohydrate (83% starch, 17% maltodextrin), and 10.5% fat (38% soybean, 62% coconut oil). The high-fat diet (D12331) consisted of 16.4% protein, 25.5% carbohydrate (51% sucrose, 49% maltodextrin), and 58% fat (93% coconut oil, 7% soybean oil). Previous studies in our laboratory (19) and others (24, 25) have confirmed that these diets result in significant DIO (increased body weight, increased body fat mass) in these mice. This induction of obesity had also been shown to be reversed in these animals by switching from high- to low-fat diet (25).

Influenza viruses and infection

Mouse-adapted influenza virus strain X-31 (H3N2) (a generous gift from David Woodland, Trudeau Institute, Saranac Lake, NY) and A/Puerto Rico/8/34 (PR8, American Type Culture Collection, Manassas, VA) were grown in the allantoic fluid of embryonated hen's eggs. Created by Edward Kilbourne (1969), influenza X-31 is a mouse-adapted recombinant influenza virus consisting of the external hemagglutinin and neuramidase (NA) proteins of A/Aichi/2/68 (H3N2) and the internal proteins of PR8 (H1N1) (26). The X-31 strain is sublethal and efficient at producing memory T cells that are able to fight a secondary A/PR/8 infection (27). For primary infection, lean and obese mice were anesthetized i.p. with ketamine/xylazine and subsequently inoculated intranasally with 300 egg infectious dose 50% live X-31 virus in 0.03 ml sterile PBS. Thirty-one days postinfection (p.i.), no X-31 virus was detected by quantitative real-time-PCR (qRT-PCR) or tissue culture infections dose 50 ($TCID_{50}$) in mouse lungs ($n = 8$) in either lean or obese mice. Mice were then challenged at 31 d after X-31 infection intranasally with a secondary infection of 5000 $TCID_{50}$ ($100\times LD_{50}$ in lean mice during a primary infection) live PR8 virus in 0.05 ml sterile PBS.

Lung histopathology

As previously described (28), lungs were removed at d 10 p.i. and perfused with Optimal Cutting Temperature Compound (Tissue-Tek, Torrance, CA) and frozen on dry ice. Frozen sections (6 μm) were stained with H&E. The extent of lung pathology was graded in a semiquantitative manner according to the relative degree (from lung to lung) of mononuclear cell infiltrate. Briefly, the scoring is on a scale from 0 to 4: 0, no inflammation; 1, mild influx of inflammatory cells; 2, increased inflammation with ~25–50% of the total lung involved; 3, severe inflammation involving 50–75% of the lung; and 4, almost all lung tissue contains inflammatory infiltrates.

Quantitation of viral titers in lungs

Lung viral titers were determined by a modified $TCID_{50}$ using hemagglutination as an endpoint, as previously described (19). Briefly, half of the right lobe of the lung was removed, weighed, and ground in 0.2 ml MEM. Samples were centrifuged at $9000\times g$ for 20 min and the supernatant was serially diluted starting at 1:10 in MEM containing 20 mg/l trypsin. Each diluted supernatant (0.1 ml) was added, in duplicate, to 80% confluent Madin-Darby canine kidney cells and incubated at 37°C for 96 h. A 0.5% suspension of human O RBC (0.05 ml) was added to each well and incubated at room temperature for 2 h. $TCID_{50}$ was determined by the method of Reed and Muench (29). Values were normalized to weight of the original tissue used.

Quantitation of lung mRNA cytokine levels

Lung samples were collected on d 0 (uninfected), 1, 2, 3, 7, and 10 p.i. and total RNA was isolated using the TRIzol method. Reverse transcription was carried out with Superscript II First Strand Synthesis kit (Invitrogen, Carlsbad, CA) using oligo (dT) primers. According to previously described methods (19), mRNA levels for murine IFN-α, IFN-β, IFN-γ, IL-6, and TNF-α, and G3PDH were determined using qRT-PCR.

Isolation of cells from the lungs and spleen

As previously described (20), lungs from lean and obese mice were removed, digested in HBSS (with calcium and magnesium) supplemented with 160 U/ml Collagenase type 1 (Worthington, Lakewood, NJ). Spleen cells were isolated in unsupplemented HBSS. Samples were processed into single-cell suspensions by mechanical agitation of a Stomacher (Seward, West Sussex, U.K.) and strained through a 40-μm nylon filter. Cells were subjected to RBC lysis using ACK lysis buffer for 5 min at room temperature, washed, counted, then subjected to analysis by flow cytometry.

Flow cytometry

At least 1×10^6 cells were stained with FITC–anti-CD44, Pacific Blue–anti-CD62L (both from eBioscience, San Diego, CA) and PerCP–anti-CD8α (BD Biosciences, San Jose, CA). $CD8^+$ T cells specific for the major epitope of the PR8 nucleoprotein were identified using a PE-labeled $D^bNP_{366-374}$ tetramer. Nonspecific tetramer staining was analyzed using an irrelevant tetramer toward HSV. Intracellular staining was performed on total lung cells from influenza-infected mice that were incubated for 4 h with GolgiPlug (BD Biosciences, San Jose, CA). Cells were then Fc-blocked with anti-CD16/CD32, surface stained, and permeabilized with Perm/Wash (BD Biosciences) for staining with allophycocyanin anti–IFN-γ. An irrelevant APC-IgG was used as a staining control. For DCs, samples were stained with FITC–anti-CD11b and eFluor 450–anti-CD11c (eBioscience). Samples were analyzed on a Cyan ADP flow cytometer (Beckman Coulter, Fullerton, CA) and data were analyzed using FlowJo software (TreeStar, Ashland, OR). Gates were set for DCs based on previous reports (20, 30, 31). Briefly, gates were set based on CD11b and CD11c expression and DCs were determined by intermediate levels of CD11b and CD11c.

Ag presentation by DCs

Using previously published methods (20), lungs from lean and obese mice infected 31 d previously with influenza X-31 were removed and processed into single-cell suspensions as described previously. Lung isolates were then pooled into groups of three mice based on diet. To isolate as many dendritic cells as possible, cells were resuspended in Ca^{2+} and Mg^{2+} free HBSS containing 10 mM EDTA and incubated at room temperature with agitation for 5 min. Cells were counted using a hemocytometer and 5×10^7 cells were used to isolate DCs from each pool using a DC enrichment kit (Invitrogen Dynal AS, Oslo, Norway). T cells were isolated from a separate group of lean and obese mice previously infected with influenza X-31. Pooled suspensions as described previously were isolated using a T cell negative isolation kit (Invitrogen Dynal AS). DCs were incubated with heat-inactivated influenza A/PR/8 at an multiplicity of infection of 2 for 2 h, followed by extensive washing to remove excess virus. DCs were plated with T cells in a 96-well plate at a DC to T cell ratio of 1:2. Samples were then incubated for 2 h at 37°C, followed by the addition of Golgi Plug (BD Biosciences) and incubated for an additional 4 h. Cells were stained with anti-CD16/CD32, FITC–anti-CD3, and PerCP-Cy5.5–anti-CD8 (eBioscience), followed by fixation and permeabilization for subsequent intracellular staining with allophycocyanin–anti-IFN-γ. Cells were run on an Accuri C6 flow cytometer (Accuri, Ann Arbor, MI) and samples were analyzed using FlowJo software (TreeStar).

Serum leptin was measured using a commercially available ELISA (R&D Systems, Minneapolis, MN).

Statistical analysis

Statistical analyses were performed using JMP Statistical Software (SAS Institute, Cary, NC). Nonparametric data were analyzed using Kruskal Wallis test (α = 0.05). Normally distributed data were analyzed by two-way ANOVA with diet and day postinfection as main effects. Student's t test was used for posthoc comparison between the dietary groups and Tukey's HSD was used for posthoc comparisons among the days p.i. Differences were considered significant at $p < 0.05$.

Results

DIO mice have increased morbidity and mortality when challenged with a secondary influenza infection

To test the effect of obesity on influenza-specific cell mediated immunity, lean (35.7 ± 0.4 g) and obese (52.2 ± 0.4 g) mice were primed with the recombinant influenza ×31 strain (H3N2), followed by a dose of influenza PR8 (H1N1) strain 4 wk later that is normally lethal in naive mice. Primary and memory T cell response experiments can be conducted with mouse-adapted X-31 and PR8 because they differ in surface protein expression, thus minimizing Ab-mediated clearance of secondary infection (32). By day 7 after PR8 challenge, 25% of the obese mice had died with no loss of lean controls (Fig. 1A).Consistent with infection, both groups lost weight after PR8 challenge, although the infection was more severe in the obese mice as indicated by the lack of weight regain (Fig. 1B). This increased severity of infection was confirmed by the increased lung pathology and viral titers (Fig. 2A–C) in obese mice.
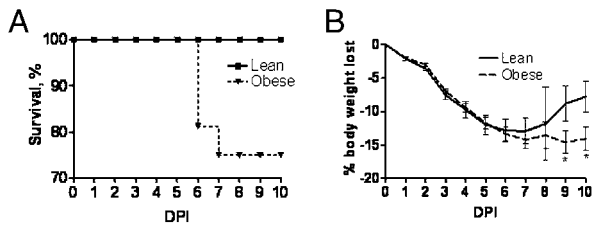
FIGURE 1. Morbidity and mortality in lean and obese mice during memory response to influenza infection. After primary infection with influenza X-31, lean and obese mice were challenged with a dose of influenza PR8 normally lethal to lean, naive mice. *A*, Lean mice had 100% survival, whereas obese mice had a 25% mortality rate by day 7 p.i. *B*, Obese mice fail to regain weight after secondary infection ($n = 12$–24 per group) $\pm$ SEM. $*p < 0.05$, lean versus obese.

DIO alters antiviral cytokine expression in the lungs after secondary challenge

Respiratory epithelial cells are a primary target of influenza virus and play an important role in the pathogenesis of influenza infection. Infection of respiratory epithelial cells with influenza virus results in activation of retinoic acid-inducible gene-I and TLR 3 that then signal to begin production of IFN-α and IFN-β (33, 34). These key cytokines function to control viral replication as well as induce both the innate response and the subsequent generation of the adaptive immune response (35–37). Concordant with our observations of primary influenza infection in obese mice (19), lean mice had a robust increase in IFN-α/β mRNA expression at day 2 postsecondary challenge; however, obese mice had no increase in IFN-α and the increase in IFN-β expression was significantly reduced versus lean controls (Fig. 3).

Obesity reduces the ability of the mice to produce a proinflammatory response postsecondary infection

Obesity leads to a low-grade inflammatory state (38) resulting in increased serum levels of TNF-α and IL-6 that are reversed by weight loss and moderate exercise (39–42). Although obesity
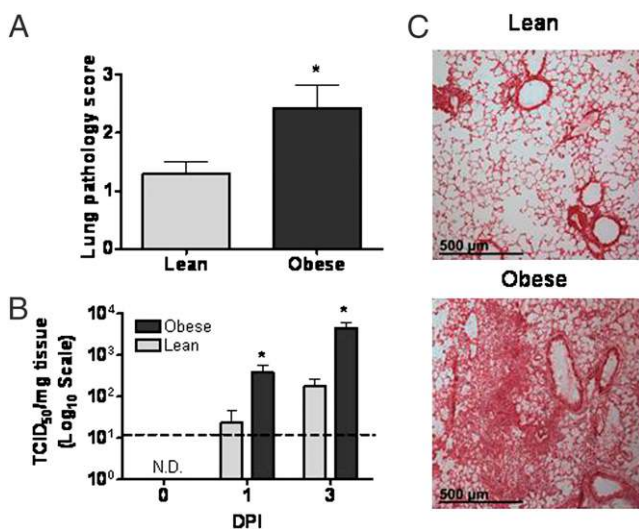

FIGURE 2. Obese mice have increased viral titer and pathology postsecondary infection. *A* and *B*, Obese mice had increased lung pathology at day 10 p.i. ($n = 10$ per group). Data are expressed as mean $\pm$ SEM. $*p < 0.05$, lean versus obese. *C*, Obese mice had a $>$10-fold increase in viral titer in the lungs at days 1 and 3 postinfection after secondary PR8 challenge. No virus was detected before PR8 infection (day 0). The dashed line indicates the limit of detection for the assay. Data are representative of three separate experiments and are expressed as $TCID_{50}$/mg lung tissue ($n = 3$–4 per group) $\pm$ SEM on a Log_{10} scale. $*p < 0.05$, lean versus obese.
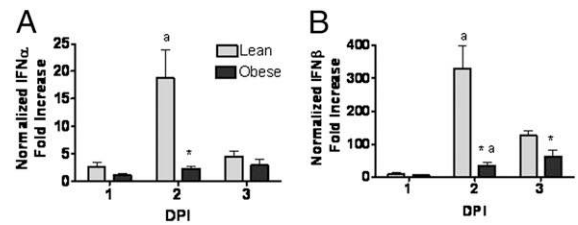

FIGURE 3. Antiviral cytokine mRNA expression in the lung after secondary infection. mRNA expression of (*A*) IFN-α and (*B*) IFN-β were measured in the lungs of lean and obese mice after secondary infection. Data are expressed by normalization to G3PDH values, then to lean value at day 0 p.i. ($n = 6$–8 per group) $\pm$ SEM. There were no significant differences in G3PDH values. $*p < 0.05$, lean versus obese; $^{a}p < 0.05$ versus day 0 of individual diet group.

leads to an inflammatory state in the serum, we also show that the lungs of obese mice were in a state of elevated inflammation even before secondary challenge (Fig. 4*A*). It is possible that because of this increased inflammatory state of the lungs, obese mice challenged with a secondary influenza infection failed to upregulate lung mRNA expression of inflammatory cytokines IL-6 (Fig. 4*B*) and TNF-α (Fig. 4*C*) resulting in a significantly blunted response relative to lean controls.

IFN-γ expression is significantly reduced in the lungs of DIO mice after secondary challenge

After a secondary challenge with influenza virus, nonproliferating memory CD8^{+} T cells in lung airways are able to respond to viral replication by generating inflammatory signals to induce the antiviral response (43, 44). These cells cannot clear virus completely, but can significantly reduce viral loads through IFN-γ production at early time points. DIO mice had significantly
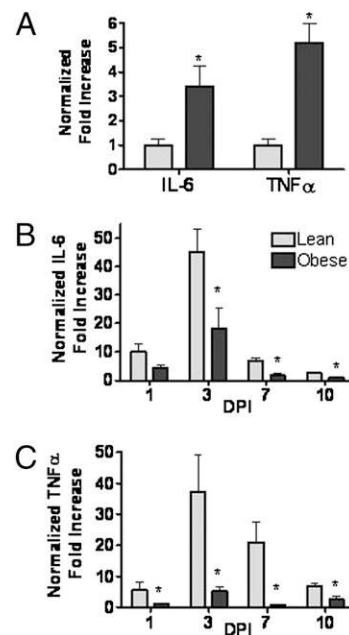

FIGURE 4. Inflammation-associated cytokine mRNA expression in the lung prior to and after secondary infection. *A*, mRNA expression of IL-6 and TNF-α were measured in the lungs of lean and obese mice before (day 31 after X-31 infection) secondary infection. Data are expressed by normalization to G3PDH values ($n = 6$–8 per group) $\pm$ SEM. mRNA expression of (*B*) IL-6 and (*C*) TNF-α were measured in the lungs of lean and obese mice after secondary infection. Data are expressed by normalization to G3PDH values, then to lean value at day 0 p.i. ($n = 6$–8 per group) $\pm$ SEM. There were no significant differences in G3PDH values. $*p < 0.05$, lean versus obese.
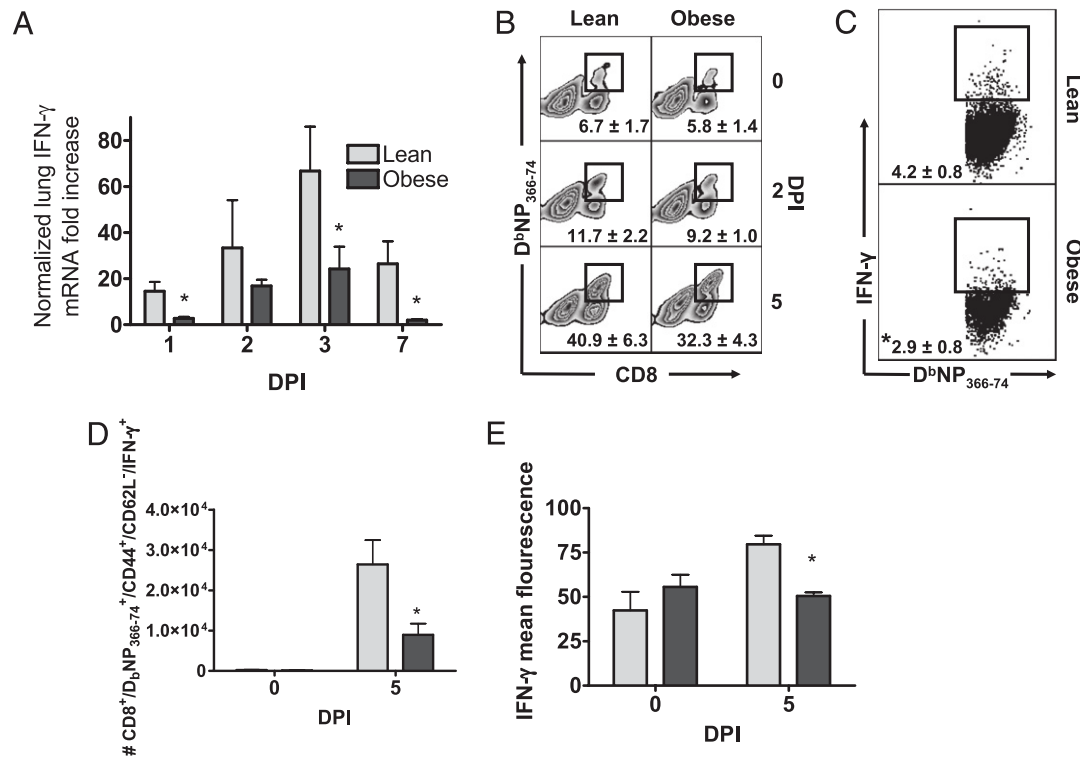
FIGURE 5. IFN-γ expression in the lung after secondary infection is reduced in obese mice. *A*, mRNA expression of IFN-γ was measured in the lungs of lean and obese mice after secondary infection using qRT-PCR. Data are expressed by normalization to G3PDH values, then to lean value at day 0 p.i. ($n =$ 6–8 per group) $\pm$ SEM. There were no significant differences in G3PDH values. $*p < 0.05$, lean versus obese. *B*, Number of influenza-specific effector memory T cells ($CD8^+/D^bNP_{366-374}{}^+/CD44^+/CD62L^-$) after secondary infection. *C*, Percent of influenza-specific memory T cells at day 5 and (*D*) number of influenza-specific effector memory T cells producing IFN-γ. *E*, Amount of IFN-γ produced by each cell using flow cytometry. Cells are gated as effector memory T cells ($CD8^+/D^bNP_{366-374}{}^+/CD44^+/CD62L^-$) unless otherwise noted. No significant differences were observed in number of total cell numbers and total $CD8^+$ T cell numbers between the lungs of lean and obese mice. Data are expressed as number or percent cells of total cells isolated and mean fluorescence intensity ($n = 4$–6 per group) $\pm$ SEM. $*p < 0.05$, lean versus obese.

reduced expression of INF-γ mRNA in the lungs at days 1, 3, and 7 postsecondary challenge (Fig. 5*A*). Although the overall percentage of memory T cells did not differ between mice (Fig. 5*B*), percentage of influenza-specific effector memory T cells ($CD8^+$ /$D^bNP_{366}{}^+/CD44^+/CD62L^-$) producing IFN-γ was reduced in DIO mice at day 5 (Fig. 5*C*). In addition, the numbers of influenza-specific effector memory T cells producing IFN-γ at day 5 postsecondary challenge was significantly reduced with less than half the total numbers responding in obese mice versus lean (Fig. 5*D*). Mean fluorescence intensity of IFN-γ production was significantly reduced in obese mice, further indicating a functional difference (Fig. 5*E*).

Diminished capacity of lung-resident memory T cells from obese mice to respond to influenza-specific Ag presentation

Despite reduced percentage (Fig. 6*A*) and number (Fig. 6*B*) of DC in the lungs of obese mice, obese DCs were able to present Ag effectively to lean memory T cells ex vivo (Fig. 6*C*). In contrast, obese memory T cells, when stimulated with lean DCs, were less able to respond to Ag presentation with a >50% reduction in IFN-γ producing cells (Fig. 6*C*).

Obesity leads to an altered serum leptin profile postsecondary infection

Leptin has been identified as an important immunomodulatory molecule for both the innate and adaptive response. Indeed, leptin is produced at high levels during inflammation and is involved in a number of immune pathways and cell types (45). As previously demonstrated (46, 47), serum leptin levels are significantly ele-

vated in DIO mice. In lean mice, leptin levels are significantly elevated postinfection; however, this response did not occur in the obese mice (Fig. 7).

Discussion

To our knowledge, this is the first time that DIO has been shown to affect the memory response to a viral infection. It is apparent that DIO results in an ineffective memory response to influenza infection impacting both memory T cell function and reducing the DC population in the lung. The protective effect of memory T cell populations against influenza infection has been shown in a number of models, including against the highly pathogenic H5N1 viruses (48). Prime-challenge with a H9N2 isolate was protective against A/Hong Kong/156/97 (49) and a double-priming with both X-31 and PR8 was protective against one of the most lethal H5N1 strains (17). Indeed, in our study, protection against a heterologous strain of influenza was observed in the lean mice with 100% survival against a lethal dose of PR8 after X-31 priming; however, obesity reduced this protective capacity. Early innate antiviral cytokines IFN-α and IFN-β were significantly delayed and reduced in the lungs of obese mice that may have contributed to the higher viral titers and increased infiltrate found in lungs of obese mice. IFN-α/β expression has also been associated with immune memory generation and function and with a direct signal from IFN-α/β required for memory T cells to survive the contraction phase of the primary response (50, 51).

During the course of an influenza virus infection, heterogeneous pools of persistent memory $CD8^+$ T cells are established. Influenza-specific $CD8^+$ T cells have been observed in both humans

FIGURE 6. Impairment in function of memory T cells from the lungs of obese mice. *A*, Percentage before secondary infection and (*B*) number of DCs in the lungs of lean and obese mice after secondary challenge. Data are expressed as percentage and number of CD11b$^+$/CD11c^{int} cells of total cells isolated ($n = 6$ per group) $\pm$ SEM. $*p < 0.05$, lean versus obese. *C*, Ability of lean and obese DCs to present Ag to lean T cells and obese T cells from lungs of influenza-experience mice. DCs and T cells were isolated from lean and obese mice at day 0 (day 31 after X-31 infection). Data are expressed as number of CD3$^+$/CD8$^+$ cells producing IFN-γ^+ as measured by flow cytometry ($n = 3$ per group per pool) $\pm$ SEM. $*p < 0.05$, lean versus obese.
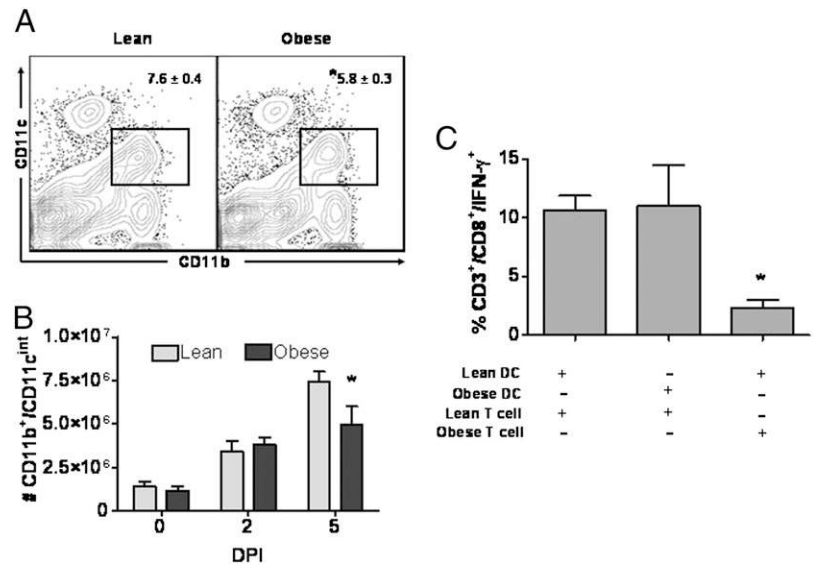
and animal models months to years after influenza infection (52–54). A significant number of memory cells can be found in secondary lymphoid organs such as the draining lymph nodes and spleen; however, as many as half of the influenza-specific CD8$^+$ memory T cells can be found in peripheral (nonlymphoid) organs (32, 52, 55). When functioning properly, populations of memory T cells serve not to prevent viral infection, but to reduce the level of viral amplification until a sustained supply of new effector cells can be generated (56). Reduction of either the function or number of these cells could lead to the inability to mount an efficient secondary response. Based on the increased morbidity and mortality seen in obese mice, along with the reduced effector function of obese influenza-specific memory T cells versus the lean controls, it is clear that DIO mice have a significantly reduced capacity to respond to a novel influenza virus strain.

The exact mechanism by which T cell memory develops remains controversial; however, the main consensus appears to be an adequate/balanced primary response. Development of a functionally protective CD8$^+$ memory response depends on the integration of multiple signals both by responding T cells and by other immune mediators such as APCs. Ag density and time of contact with APCs, levels of inflammatory cytokines, and overall primary effector response all play a role in programming the development of effective memory T cell responses (21). The reduced protective capacity of the memory response in obese mice could be caused by defects in a single or combination of factors during the primary response.

APCs depend on inflammatory signals for potent stimulation of T cell responses; however, it has become clear that T cells also receive signals from inflammatory cytokines. In the obese host, inflammation is altered during infection. We have shown inflammatory signals are delayed but increased in obese mice during primary influenza infection (19). The amount of inflammatory signals received appears to balance effector and memory generation in primary CD8$^+$ T cells. Higher levels of inflammatory cytokines indicate the need for a stronger initial response, which may lead to lowered memory generation (57). During primary influenza infection, we observed an increased Ag-specific T cell response (20), potentially resulting in decreased memory T cell generation. This chronic inflammatory state, as well as greater expression of inflammatory mediators during the primary infection, could tip the balance of memory cell generation to a "too much, too late" scenario. This scenario could push development toward greater generation of primary influenza-specific effector

T cells resulting in diminished numbers or function in memory cell precursors.

CD8$^+$ T cell responses have evolved to specifically eliminate pathogens and to protect against reinfection (58, 59). During infection with PR8, lack of CD8$^+$ cells results in decreased viral clearance and eventual morbidity (60, 61). Ag-specific memory T cells are protective because they are present in higher numbers than naive precursors and respond rapidly on re-encounter with pathogen (62). Secondary influenza-specific T cell responses arise ~2 d faster than the primary response and have greatly increased activity and effector function (17). After a secondary challenge with influenza virus, memory CD8$^+$ T cells in the lung airways respond to initial viral loads by generating signals, such as IFN-γ, to induce the antiviral response (43, 44). The protective value of CD8$^+$ T cell memory cells is strongly correlated to the ability to exert effector function at the site of infection (63). Reduction in the relative number of memory T cells in the lung airways has been correlated to a decline in the overall recall response to viral challenge (52, 64). DIO mice had decreased overall IFN-γ mRNA expression in the lung, as well as decreased percentage and overall number of influenza-specific effector memory T cells producing IFN-γ postsecondary challenge. These data indicate a significant reduction in the ability of influenza-specific T cells in the lungs of obese mice to respond to a heterogeneous viral strain.

The decreased production of IFN-γ by memory T cells during secondary infection in DIO mice may be due to impairment in DC function, as we previously demonstrated during primary influenza infection in DIO mice (20). Interestingly, we found that in contrast to a primary influenza infection, DCs from DIO mice were able to efficiently present Ag to lean and obese memory T cells. There is
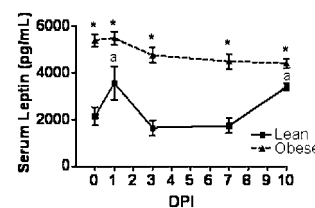

FIGURE 7. Serum leptin concentrations for lean and obese mice after secondary challenge. Serum leptin levels measured by ELISA. Data as expressed as pg/ml ($n = 6$–8 per group) $\pm$ SEM. $*p < 0.05$, lean versus obese; $^a p < 0.05$ versus day 0.

some evidence that DC interaction is important for activating the CD8^{+} memory response; however, requirements for costimualtion by DCs are considerably lower that that of naive precursors that may explain why obese DCs were able to stimulate lean memory T cells (65, 66). In addition, reduction in the number of influenza-specific T cells may indicate an impairment of memory T cells to migrate to the obese lung microenvironment. Tissue-specific homing of memory cells allows for increased efficiency of adaptive response by providing increased numbers of Ag-specific T cells at the anatomical site most likely for reinfection (67). A similar situation could be associated with the reduction in the number of DCs from the obese lung versus lean controls.

Although the mechanistic link between obesity and diminished immune memory is not clear, a main factor that ties together obesity, inflammation, and immune cell function is leptin resistance associated with the obese state. Leptin is recognized as an important mediator of immune function (68) and leptin expression is increased proportionately with adipose tissue mass, resulting in increased circulating levels in obesity (69). This chronic elevation appears to cause a state of leptin resistance in obese mice where leptin signaling is attenuated despite increased circulating levels (46). Leptin signaling is important for virtually all parts of the innate immune response, including antiviral (IFN-α/β) cytokine expression, proinflammatory IL-6, and TNF-α expression, and activation and stimulation of monocytes, DCs, and macrophages (70, 71). Leptin also modulates adaptive immunity by increasing DC function and stimulatory capacity, promoting a Th1 response and enhancing T cell survival and proliferation (72–75). Leptin resistance has been found to negatively impact T cell responses as well as DC maturation and survival in obese mice (73, 76, 77). In this experiment, we have observed effects consistent with leptin resistance. Indeed, serum leptin was significantly increased in obese mice before secondary challenge and did not show an infection associated response as seen in the lean controls. Therefore, leptin resistance in obese mice may contribute to the reduced protective capacity of the memory response to secondary influenza infection.

The ability to generate functional memory T cells, either during primary infection or by vaccination, has proven to be protective against potentially lethal influenza strains exhibiting completely different surface Ags (17). The Centers of Disease Control and Prevention and World Health Organization have declared the currently circulating novel H1N1 influenza strain to be pandemic and obesity appears to be an independent risk factor for illness severity (18). In this paper we have demonstrated that increased morbidity and mortality during a secondary influenza infection in DIO mice is due to impairment in the ability to generate and maintain functional influenza-specific memory T cells. This finding, if applicable to a human obese population, has significant public health implications, including current vaccine strategies which may be less effective in an increasingly obese population.

Acknowledgments

Disclosures

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