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BRIEF CUTTING EDGE REPORT

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Defective interferon amplification and impaired host responses against influenza virus in obese mice

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Abstract

Objective: Obesity is a major risk factor that increases morbidity and mortality upon infection. Although type I and type III interferon (IFN)-induced innate immune responses represent the first line of defense against viral infections, their functionality in the context of metabolic disorders remains largely obscure. This study aimed to investigate IFN responses upon respiratory viral infection in obese mice.

Methods: The activation of IFNs as well as IFN regulatory factors (IRFs) upon H3N2 influenza infection in mice upon high-fat-diet feeding was investigated.

Results: Influenza infection of obese mice was characterized by higher mortalities. In-depth analysis revealed impaired induction of both type I and type III IFNs as well as markedly reduced IFN responses. Notably, it was found that IRF7 gene expression in obese animals was reduced in homeostasis, and its induction by the virus was strongly attenuated.

Conclusions: The results suggest that the attenuated IRF7 expression and induction are responsible for the reduced expression levels of type I and III IFNs and, thus, for the higher susceptibility and severity of respiratory infections in obese mice.

INTRODUCTION

Infections of the respiratory tract represent a serious health problem. Obesity is a major risk factor for these infections and it contributes to their severity. As a consequence of the multifaceted nature of obesity, the pathogenesis of various respiratory infections is altered, including infections with influenza virus (1) and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (2).

Upon infection, the induction of interferon (IFN) responses represents the first line of defense (3). IFN induction is crucially mediated by various transcription factors, including IFN regulatory factors (IRFs) (4). Respiratory viruses such as influenza virus and SARS-CoV-2 infect alveolar epithelial cells and initiate IFN responses therein. These cells sense specific viral molecular patterns and activate nuclear factor-κB (NF-κB) and IRF3, resulting in induced expression of early IFNs of type I (IFN-α4, IFN-β) and type III (IFN-λ) (5). IFNs are secreted and they initiate a highly effective antiviral program in an autocrine and paracrine manner. Upon signaling via the cognate receptors, the IFN-stimulated gene factor 3 (ISGF3) protein complex is formed. The complex consists of IRF9 and phosphorylated signal transducer and activator of transcription 1 (STAT1) and STAT2 proteins. More than 100 IFN-stimulated genes (ISGs) are induced, impairing various steps in the viral life cycle. IRF7 is one of these ISGs. Enhanced expression of IRF7 boosts the production of IFNs after an infection. These IFN superproducer cells, together with plasmacytoid dendritic cells, which constitutively express high levels of IRF7, are responsible for most of the systemically produced type I and III IFNs (6). It has been reported that members of the IRF family, particularly IRF3, IRF7, and IRF9, are dysregulated in the livers of obese mice and contribute to metabolic disorders (7,8,9); however, the consequences for infection remained unclear.

Although type I and III IFNs depend on the same triggers and transcription factors and induce overlapping antiviral activities (3,5), their biological functions are considered to be different because the receptor for type I IFN is ubiquitously expressed, whereas the type III IFN receptor is primarily expressed in epithelial cells of the lungs and the intestinal tract (10).

Previous studies have suggested that obese mice, as well as people with obesity, show alterations in cytokine profiles and in response to infections (11,12,13). However, molecular links between obesity, its high prevalence as a comorbidity of respiratory viral infections, and, in particular, the relevance of the IRF7 amplification loop remain to be elucidated.

In this study, we elucidated whether and how obesity affects the innate immune response to influenza virus infection. We observed that obese mice showed lower expression levels of both type I and type III IFNs and higher mortalities, accompanied with reduced expression levels of prototypical antiviral ISGs. Notably, constitutive and induced levels of IRF7 were also significantly reduced in the lungs of obese mice, suggesting a crucial role of this amplifier in impairing a protective IFN response upon respiratory infection.

Study Importance

What is already known?

- Obesity is a major risk factor for the severity of viral infections.
- Type I and type III interferon (IFN) responses are key to control early viral infections.
- Steady-state levels of IFN regulatory factors (IRFs) are deregulated in the liver of obese mice.

What does this study add?

- Obesity leads to an impaired induction of type I and type III IFNs upon influenza virus infection in lung tissue.
- IRF7 upregulation upon infection is lower in obese animals, explaining the impairment of IFN production through attenuation of the positive feedback loop.

How might these results change the direction of research or the focus of clinical practice?

- This study, together with recently published work on severe acute respiratory syndrome coronavirus 2 and other virus infections, will induce a future focus on IRF7 and its boosting of interferon production in individuals with obesity and other metabolic disorders.

METHODS

Mice

IFN-$\beta^{+/\Delta\beta\text{-luc}}$ mice (IFN-β-Luc, on C57BL/6 background) (14) were maintained in individually ventilated cages under specific pathogen-free conditions. Male mice aged 6 to 8 weeks received a 45% fat (high-fat diet) or 13% fat diet (standard diet) (EF D12451 and EF D12450B, Ssniff, Soest, Germany) for 16 weeks with free access to sterile water and diet until the end of the experiment, as described previously (15).

Statistical evaluation

All data are displayed as mean (SD). Survival curves were compared using a log-rank (Mantel-Cox) test. Statistical significance of differences in quantitative reverse transcription-polymerase chain reaction and luciferase levels was calculated by Student *t* test, Mann-Whitney *U* test, or one-way ANOVA with Tukey multiple comparison test (GraphPad Prism version 5.1, GraphPad Software, San Diego, California).

For all other methods and details, see online Supporting Information.

RESULTS

Deteriorated metabolic status of obese mice upon influenza infection

We studied the impact of obesity-induced metabolic changes on the innate immune response upon influenza virus infection in male IFN-β-Luc mice. These mice carry the luciferase reporter gene on one allele of the IFN-β gene and allow the monitoring of IFN-β promoter activation without compromising innate immune responses (14). Upon feeding with high-fat diet for 16 weeks, IFN-β-Luc mice displayed changes associated with obesity, such as increased body weight, hepatic steatosis with increased numbers of lipid droplets, and increased relevant metabolic markers (Supporting Information Figure S1). In order to evaluate whether obesity affects viral susceptibility, we intranasally infected obese and control mice with 10

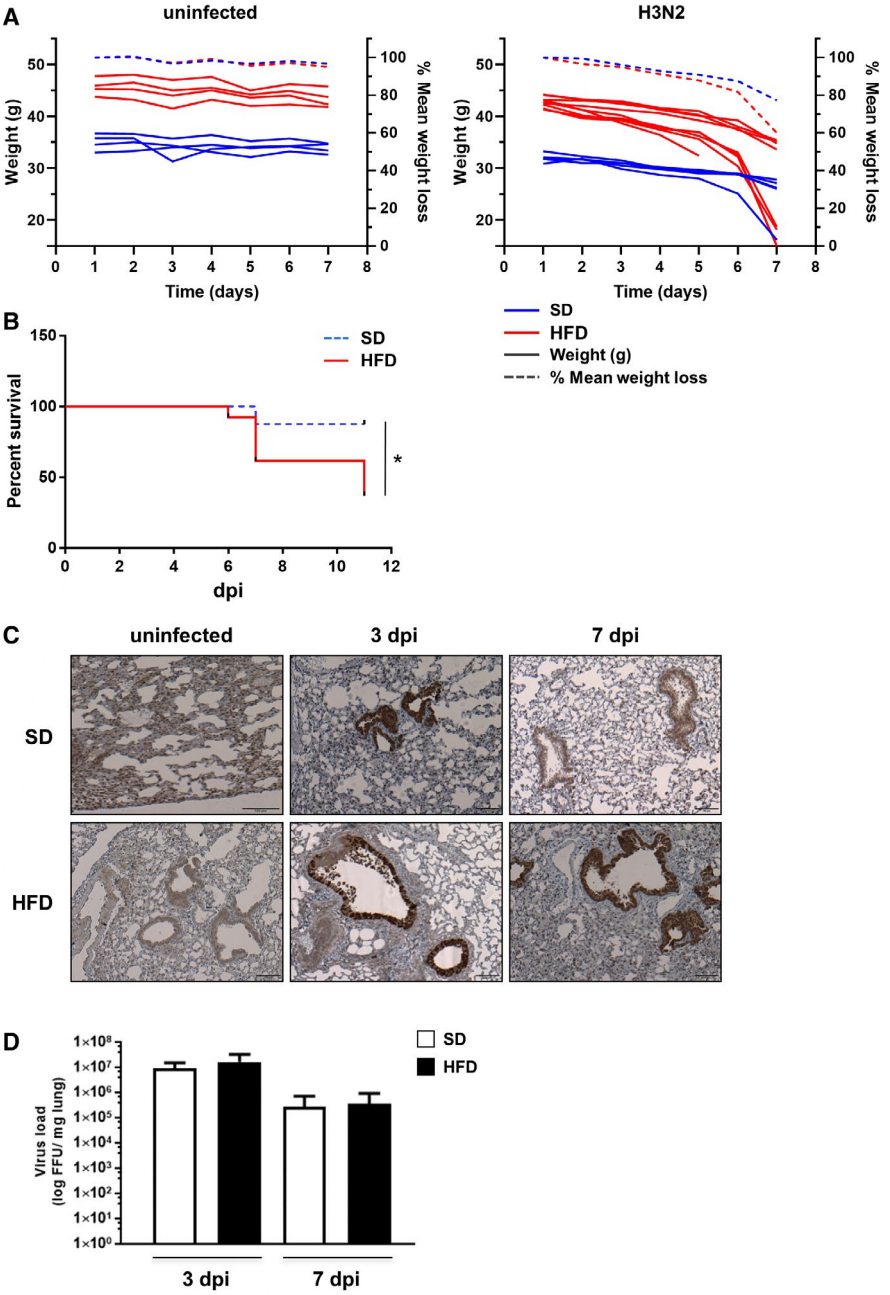

FIGURE 1 H3N2 influenza infection in obese IFN-β-Luc mice. (A) The body weight of individual SD and HFD-fed IFN-β-Luc mice upon infection with 10 focus forming units of H3N2 as well as mean percentage of weight (n = 4-7 per group). (B) Percentage of SD/HFD mice that did not reach the criteria for euthanasia after H3N2 infection. The experiment was performed with 12 HFD-treated and 8 SD-treated animals. *$p \leq 0.05$. (C) SD/HFD-treated mice were intranasally infected with 10 focus forming units of influenza virus strain H3N2. Lung sections were stained for influenza virus nucleoprotein at day 3 and day 7 post infection. Representative pictures are shown. (D) Viral titers were determined in lungs from infected SD/HFD mice at day 3 and day 7 post infection. dpi, days post-virus infection; HFD, high-fat diet; IFN, interferon; SD, standard diet [Color figure can be viewed at wileyonlinelibrary.com]

focus forming units of influenza A virus H3N2. Obese mice displayed a higher severity of illness as reflected by extended weight loss, with more animals reaching the criteria for euthanasia (Figure 1A,B). Staining of lung tissue sections for influenza virus nucleoprotein confirmed infection of epithelial cells around the bronchioles (Figure 1C). Comparable viral titers were observed in the lung tissue of obese and lean mice, demonstrating that virus infection was efficient in both conditions (Figure 1D). Notably, serum levels of alanine transaminase (ALT), aspartate transaminase (AST), and cholesterol were elevated in obese mice (Supporting Information Figure S2), indicating that infection further deteriorates the metabolic status of the animals.

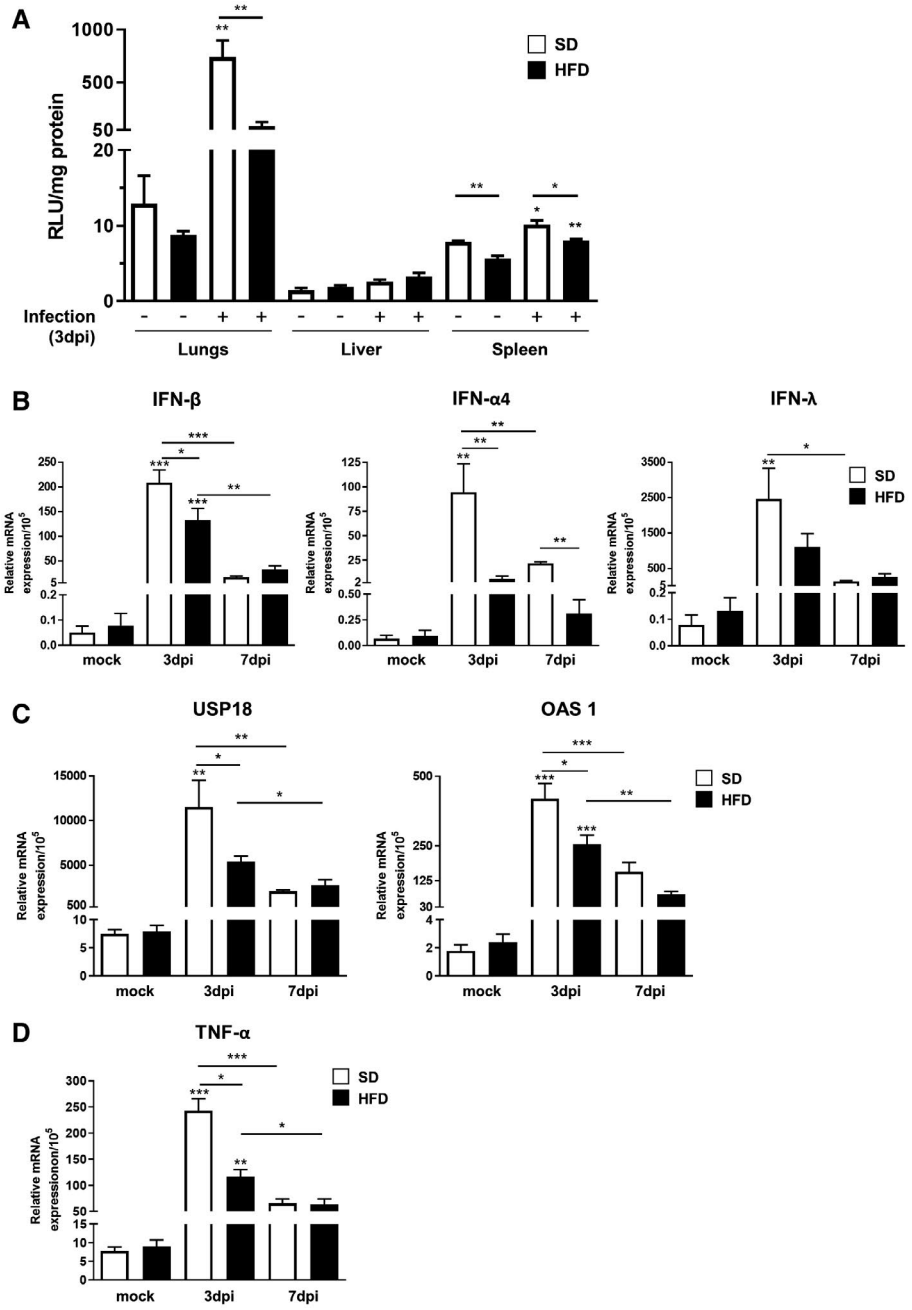

FIGURE 2 Type I and type III IFN dysregulation upon influenza virus (H3N2) infection. (A) *Ex vivo* analysis of luciferase expression in various tissues of lean and obese IFN-β-Luc mice. Tissues were isolated from mock-treated mice and from H3N2-infected animals at day 3 post infection. Luciferase activity was calculated per milligram of tissue. Four animals per group were used. (B) Relative mRNA expression levels of IFN-β, IFN-α4, and IFN-λ were determined in the lung tissue of infected and noninfected obese and lean mice (n = 4 animals per group). (C,D) The mRNA expression levels of USP18, OAS1, and TNF-α were determined at day 3 and day 7 post infection (n = 4 animal per group). For the sake of clarity, significant differences between groups and respective controls are indicated by stars above bars, whereas comparisons between different groups are indicated by a line. $^{*}p \leq 0.05$, $^{**}p \leq 0.01$, $^{***}p \leq 0.001$. dpi, days post-virus infection; HFD, high-fat diet; IFN, interferon, OAS1, 2′-5′-oligoadenylate synthetase 1; RLU, relative light units; SD, standard diet; TNF-α, tumor necrosis factor alpha; USP18, ubiquitin-specific peptidase 18

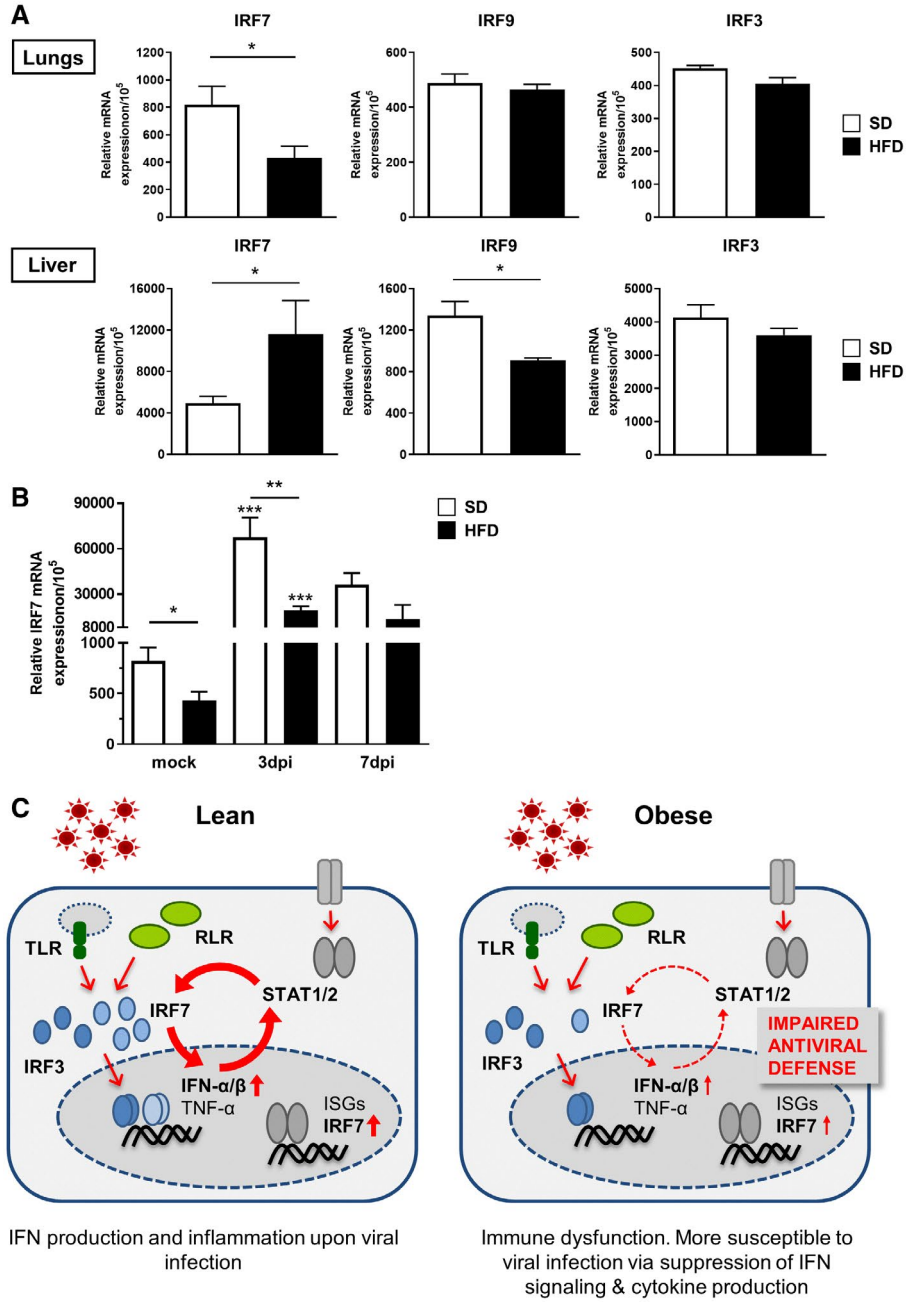

FIGURE 3 Expression of IFN regulatory factors IRF3, IRF7, and IRF9 in SD/HFD-treated IFN-β-Luc mice. (A) Steady-state mRNA levels of IRF3, IRF7, and IRF9 were detected in the lungs and liver of noninfected SD/HFD IFN-β-Luc mice (n = 4 animals per group). (B) The mRNA expression level of IRF7 in lungs was determined at day 3 and day 7 post infection (n = 4 animals per group). (C) Mechanistic representation of proposed IRF7 regulation in influenza-infected obese and lean mice. For the sake of clarity, significant differences between groups and respective controls are indicated by stars above bars, whereas comparisons between different groups are indicated by a line. $^{*}p \leq 0.05$, $^{**}p \leq 0.01$, $^{***}p \leq 0.001$. HFD, high-fat diet; IFN, interferon; IRF, interferon regulatory factor; ISG, IFN-stimulated gene; RLR, Rig-like receptor; STAT1/2, signal transducer and activator of transcription; SD, standard diet; TLR, Toll-like receptor; TNF-α, tumor necrosis factor alpha [Color figure can be viewed at wileyonlinelibrary.com]

Type I and III IFNs are reduced in influenza-infected obese mice

We assessed luciferase reporter expression at day 3 post infection. Luciferase induction was restricted to lungs, confirming local infection. Significantly lower luciferase activities were detected in obese mice (Figure 2A), and lower IFN-α and IFN-β mRNA levels were observed in lungs of H3N2-infected obese mice compared with control mice, indicating an impaired induction of type I IFNs. Similarly, IFN-λ levels were decreased; however, the reduction did not reach statistical significance (Figure 2B). In order to monitor the antiviral status, we determined the mRNA levels of the ISGs ubiquitin-specific

peptidase 18 (USP18) and 2′-5′-oligoadenylate synthetase 1 (OAS1; Figure 2C). Both genes were induced upon infection; however, obese mice displayed lower mRNA levels on day 3 post infection. We evaluated whether the lower antiviral response in obese mice is accompanied with changes in the inflammatory response, which follows type I IFN activation. Notably, mRNA levels of the proinflammatory cytokine tumor necrosis factor alpha (TNF-α) were decreased in obese mice on day 3 (Figure 2D). Thus, obese mice showed lower induction of the antiviral IFN genes and an impaired antiviral defense.

IRF7 is dysregulated in lungs of obese mice

Driven by earlier work that has addressed the regulation of IFN genes in the lungs of obese mice, we analyzed the constitutive expression level of IRF7 in the lungs. Strikingly, the expression level of IRF7 was significantly reduced in the lungs of obese mice, whereas unaltered levels of IRF9 and IRF3 were detected in both groups (Figure 3A). At the same time, in the liver of obese mice, the steady-state expression of IRF7 was increased. We observed the opposite effect for IRF9 expression, whereas IRF3 levels showed no difference, confirming previous observations (7,8,9).

Owing to the fact that IRF7 is regulated by type I and type III IFNs, we asked whether the expression of IRF7 is altered upon infection. Therefore, we evaluated the IRF7 levels in lungs of H3N2-infected animals. Notably, IRF7 mRNA levels significantly reduced in infected obese mice at day 3 post infection (Figure 3B), indicating a pronounced dysregulation of this transcription factor.

DISCUSSION

Patients with metabolic diseases are considered immunocompromised and particularly vulnerable to opportunistic infections (16). Various immunological processes are modulated in patients with obesity, with versatile and complex underlying mechanisms (17). Of note, certain metabolic conditions such as elevated lipid levels can foster viral replication but can also cause inflammation. In line with this, statins have been shown to impair viral replication and increase survival in a mouse influenza model (18) and are considered to improve therapy in the course of SARS-CoV-2 infections (19).

Respiratory viral infections are characterized by induction of IFNs in the lungs, which is considered to contribute to viral clearance (20). Notably, reduced type I IFN responses were reported in patients and mice with obesity (11,12). In the present study, we demonstrate that influenza-infected obese mice show reduced induction of IFN-α and IFN-β, as previously demonstrated (12). Moreover, we demonstrate a lower steady state as well as reduced induction levels of IRF7, which is crucial for boosting IFN responses (6). We also observed a tendency toward reduced levels of IFN-λ, which represents the most relevant early IFN in epithelial cells (5). These observations are in agreement with a recent study on influenza infection in human epithelial cells which demonstrated that the *de novo* synthesis of IRF7 is required for the second phase induction of both type I IFNs and also IFN-λ (21).

Previously, it has been shown that various IRFs are modulated in conditions of overnutrition stress in liver: whereas IRF3 and IRF9 play a protective role in fatty liver disease and hepatic insulin resistance, IRF7 contributes to hepatic steatosis (7,8,9). However, the relevance of IRFs for IFN responses in conditions of metabolic disorder remained unclear. We demonstrated that, in lungs of obese mice, homeostatic levels of IRF7, but not of IRF3 and IRF9, are changed, suggesting a particular relevance of this transcription factor in conditions of obesity.

The role of IRF7 in influenza infection in lean mice is complex and controversial. Despite the fact that deficiency of IRF7 increases the susceptibility to deadly influenza A virus infection in both humans and mice (22,23), a recent report indicates that attenuation rather than the abolishment of IRF7 activity in local infectious sites of trachea and lungs could alleviate H1N1 influenza A virus-induced acute lung injury (24). The study demonstrates that a lower IRF7 activity can affect neutrophil infiltration, but that it does not change the viral load in lungs of infected mice. On the other hand, IRF7 is the master regulator of the IFN-α genes (Figure 2B; (22)), and, in line with this, we observed a stronger reduction of IFN-α4 expression in obese mice compared with the other IFN subtypes. Therefore, we cannot rule out that ,besides IRF7, the altered ratios of the individual IFNs could also contribute to the increased severity of influenza infection in obese mice.

Recent work on the susceptibility of SARS-CoV-2- and influenza-virus-infected individuals toward severe disease correlates with genetic defects in the IFN system, including IRF7 mutations (25,26), although recovery from infections with less severe lung pathogens does not dependent on IRF7 (23). This suggests a dominant role of IRF7 in particular for the severe infections.

Therefore, the IRF7-mediated autoregulatory loop that boosts IFN production and thereby protects the body from respiratory infection is of extraordinary importance. Its vulnerability in metabolic disorders is an important disease determinant. Together, our results suggest that the severely impaired host response in patients with metabolic disorders is a consequence of both the lower induction of type I and type III IFNs and a strongly impaired positive feedback amplification (Figure 3C). After proving this correlation in patients with obesity, it might provide novel targets for therapeutic interventions.O

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CONFLICT OF INTEREST

The authors declared no conflict of interest.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the Supporting Information section.

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