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Leptin signaling maintains autonomic stability during severe influenza infection in mice

Andrés R. Muñoz-Rojas,[1,2] Adam C. Wang,[1,3] Lisa E. Pomeranz,[3] Elizabeth L. Reizis,[3] Heather W. Stout-Delgado,[4] Ileana C. Miranda,[5] Krishnan Rajagopalan,[6] Tadiwanashe Gwatiringa,[6] Roger R. Fan,[6] Ahmad A. Huda,[3] Neha Maskey, [3] Roseline P. Olumuyide,[3] Aryan S. Patel,[6] Jeffrey M. Friedman,[3] Diane Mathis,[2] and Kartik N. Rajagopalan [6]

Address correspondence to:

Jeffrey M. Friedman, 1230 York Ave., New York, New York 10065, USA. Phone: 212.327.7442; Email: friedj@rockefeller.edu

Diane Mathis, 77 Avenue Louis Pasteur, Boston, Massachusetts 02115, USA. Phone: 617.432.7742; Email: dm@hms.harvard.edu

Kartik N. Rajagopalan, 5323 Harry Hines Blvd., Dallas, Texas 75390, USA. Phone: 214.645.7988; Email: kartik.rajagopalan@utsouthwestern.edu

No conflict of interest exists.

[1] Contributed equally to this work.
[2] Department of Immunology, Harvard Medical School, Boston, Massachusetts, USA
[3] Laboratory of Molecular Genetics, Howard Hughes Medical Institute, Rockefeller University, New York, New York, USA
[4] Department of Medicine, Division of Pulmonary and Critical Care Medicine, Weill Cornell Medicine, New York, New York, USA
[5] Laboratory of Comparative Pathology, Weill Cornell Medicine, Memorial Sloan Kettering Cancer Center, and The Rockefeller University, New York, New York, USA
[6] Department of Internal Medicine, Division of Pulmonary and Critical Care, UT Southwestern Medical Center, Dallas, Texas, USA

Mice with deficient hypothalamic leptin signaling have increased susceptibility to influenza (1). To better understand the role of leptin in the response to influenza infection, we infected leptin deficient *ob/ob* mice with influenza and found that they suffered from high mortality, which was completely prevented by leptin.

While diet-induced obese (DIO) mice have fasting hyperglycemia and weight comparable with those of *ob/ob* mice (Supplemental Figures 1A and B), inoculation of 100 PFU of influenza A/PR/8/34 caused 100% mortality in *ob/ob* mice (Figure 1A) but not DIO mice, indicating that leptin deficiency rather than adiposity and metabolic dysregulation was the major contributor to increased mortality in *ob/ob* mice. Chronic leptin supplementation completely reversed mortality (Figure 1B) even though these mice ate less food and lost more weight (Supplemental Figures 1C and 1D) while acute leptin supplementation prior to infection had no rescue effect (Supplemental Figures 1H, 1I, 1J).

We measured serum levels of cytokines in *ob/ob* mice ("ob-Ctrl") and mice supplemented with 75 ng/hr of leptin ("ob-Ctrl") after infection. Certain anti-viral cytokines and chemokines were elevated in ob-Leptin mice one day after infection and normalized by day four (Figure 1C, D, E); others showed no difference (Supplemental Figure 2A).

Whole-tissue sequencing (RNAseq), differential gene-expression analysis, and hierarchical clustering on the RNA from the lungs and spleens of ob-Ctrl and ob-Leptin mice revealed several differences (Supplemental Figure 3). Gene-Set Enrichment Analysis (GSEA) showed two gene clusters in lung that were expressed at higher levels in ob-Leptin mice and were classified as "immunologic activation" and "extracellular

matrix pathways" (Figure 1F). Two gene clusters in spleen had higher expression levels in ob-Leptin mice and were classified as "T cell activation," "immunologic activation," and "antigen processing pathways" (Figure 1G). These results suggest that leptin increased immunologic activation early in infection, which normalized by day seven.

We performed immunophenotyping eight days after infection and found a marked increase in both the spleen size and number of CD45+ cells in ob-Leptin mice (Figure 1H, I), including an increase in the proportion of B cells and a concomitant decrease in the proportion of T-cell receptor β (TCRβ+) cells (Figure 1J). Th1 cells were increased in ob-Leptin mice (Figure 1K), indicating an improved Th1 anti-viral systemic response. Immunophenotyping of the lung showed that the proportions of conventional dendritic cells (cDC2s) and Th1 cells were increased in the ob-Leptin group while the proportions of macrophages and Tregs in the lung were decreased (Figure 1L). These differences were accounted for by increases in the number of DCs, T conventional (Tconv) cells, Th1 cells, B cells, and total CD45+ cells in ob-Leptin mice (Figure 1M). These analyses are consistent with an elevated Th1 anti-viral response in ob-Leptin mice.

Despite the marked difference in mortality, there was neither a difference in lung (Figure 1N), nor trachea (Supplemental Figure 4C), nor heterogeneity of lung injury (Supplemental Figure 4D), nor oxygen saturation (Figure 1Q) between the groups. Despite a better Th1 antiviral response in ob-Leptin mice, ob-Ctrl mice had no difficulty clearing the virus, and lung and serum viral titers were similar between the two groups (Figures 1O and 1P). In addition, blockade of type I interferon signaling in ob-Leptin mice did not reverse survival (Supplemental Figure 2B), indicating that leptin's effects might not be due to augmentation of immune function. Instead, leptin treatment

prevented profound bradycardia and hypothermia that was seen after viral infection in ob-Ctrl mice (Figures 1R and 1S).

Leptin deficiency caused a profoundly increased susceptibility to influenza infection, which was completely reversed with chronic physiologic leptin replacement prior to infection. While ob-Ctrl mice had deficient Th1 responses, they were equally capable of clearing virus as the treated group. However, untreated animals developed profound bradycardia and hypothermia prior to death, which was prevented by leptin treatment. Leptin is known to stimulate hypothalamic neurons to modulate sympathetic nerve fibers and control heart rate and temperature (2). Our findings raise the possibility that leptin signaling is implicated in preserving autonomic function after severe viral infection.

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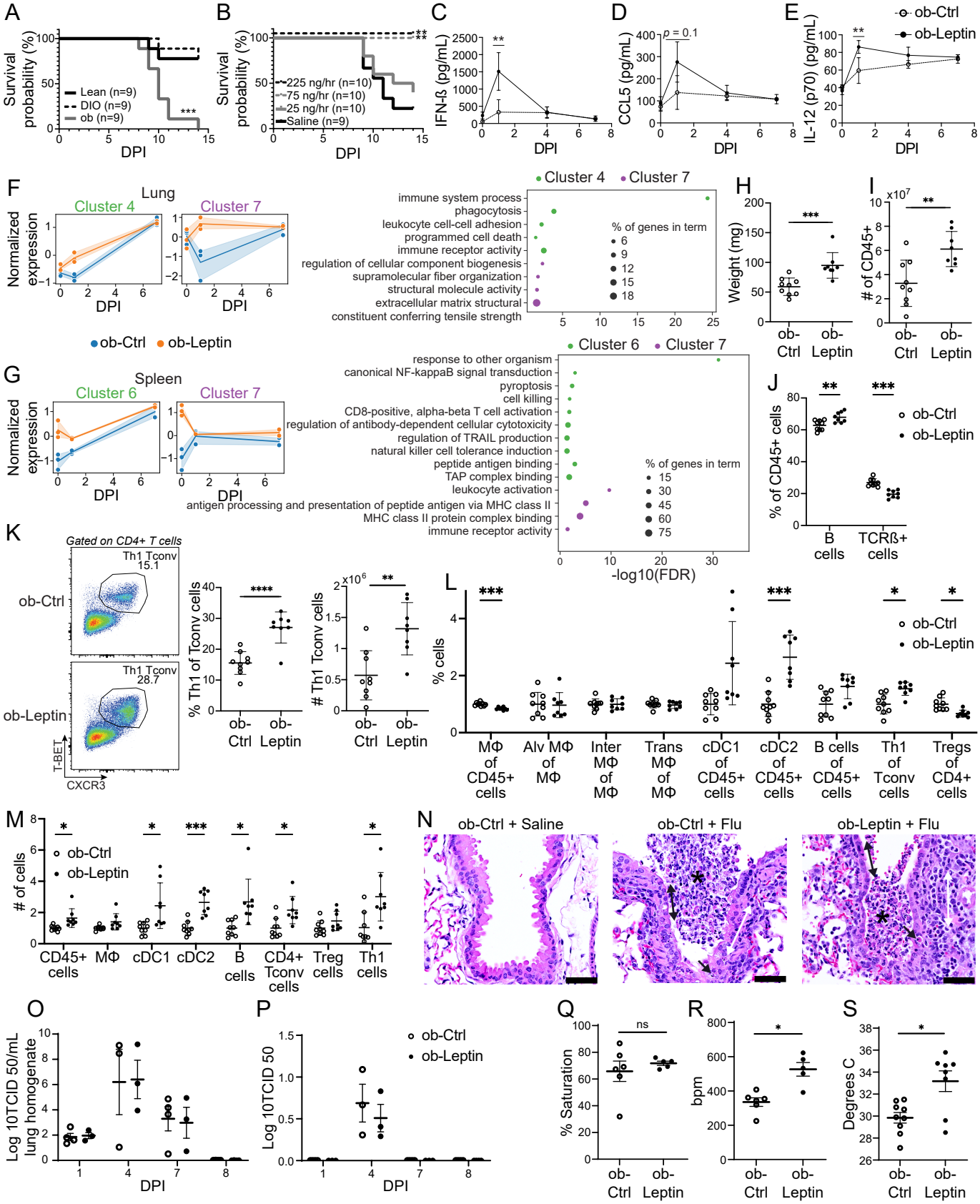
Figure 1. Leptin supplementation maintains autonomic stability during severe influenza infection. (A) Kaplan-Meier curve of influenza A infected wild-type lean and DIO mice and *ob/ob* mice and (B) influenza A infected *ob*/*ob* mice supplemented with leptin. (C-E) Serum levels of (C) IFN-ß (D) CCL5 and (E) IL-12. (F-G) Whole-tissue RNA seq of (F) lung and (G) spleen. Average normalized expression values for chosen gene clusters across time (left), and functional enrichment analysis for each cluster (right). (H) Weight, (I) number of immunocytes, and (J) proportion of cell types in spleen 8 days after infection. (K) Representative flow cytometry plots (left) and quantification (right) of Th1 cells in spleen. (L-M) Immunophenotyping of lung 8 days after infection, showing (L) proportions and (M) numbers of different cell types. (N) H&E staining of lungs. Normal uninfected tissue (left). Seven days post infection, both Ob-Ctrl infected (middle) and ob-Leptin infected (right) tissues show attenuation of the epithelium (black double-headed arrows), necrotic epithelial cells (black arrows), peribronchiolar infiltrates of immune cells (white double-headed arrows), and luminal debris (black asterisk). Magnification: 20X objective. Scale bar: 40μm. (O-P) Viral titers of (O) lung homogenates (P) and serum. (Q) Oxygen saturation (R) heart rate, and (S) core temperature of *ob*/*ob* mice 7 days after infection. OB, *ob/ob*, DIO, diet-induced obesity; DPI, days post-infection; ctrl, control; Mθ, macrophages; TCRß, T cell receptor b; Alv Mθ, alveolar macrophages; Inter Mθ, interstitial macrophages; Trans Mθ, transitional macrophages; cDC, conventional dendritic cells; Tconv, T conventional cells; Treg, regulatory T cells; TCID, tissue culture infectious dose; bpm, beats per minute; *, $p<.05$; **, $p<.01$; ***, $p<.005$; ****, $p<.001$ per Student's T-test with error bars representing SEM.



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