Leptin deficiency in vivo enhances the ability of splenic dendritic cells to activate T cells

Oscar Ramirez and Kristine M. Garza

Department of Biological Sciences and Border Biomedical Research Center, University of Texas at El Paso, El Paso, TX 79968-0519, USA

Correspondence to: K. M. Garza; E-mail: kgarza@utep.edu

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Abstract

Leptin is a pleiotropic adipokine that is critical for regulating food intake and energy expenditure and also participates in functions of the immune system, including those of antigen-presenting cells. Here, we assess the effect of leptin deficiency on the function splenic dendritic cells (sDC). sDC from leptin-deficient mice (Lep<sup>ob</sup>) were evaluated ex vivo for phenotype, ability to respond to inflammatory stimuli, to acquire and process antigens and to activate T cells. The data show that Lep<sup>ob</sup> sDC express activation markers similar to controls and respond similarly to LPS activation or anti-CD40 cross-linking. In addition, antigen acquisition and processing by Lep<sup>ob</sup> sDC was similar to controls. However, Lep<sup>ob</sup> sDC elicited higher production of IFN-γ in mixed lymphocyte reactions and increased production of IL-2 by antigen-specific T-cell hybridoma relative to controls. To assess Lep<sup>ob</sup> sDC activation of T cells in vivo, Lep<sup>ob</sup> and control mice were infected systemically with Mycobacterium avium. Lep<sup>ob</sup> mice were significantly better at neutralizing the infection as measured by splenic bacterial load over time. This was mirrored with an increased percentage of activated T cells in M. avium-infected Lep<sup>ob</sup> mice. Thus, although no changes were detected in sDC phenotype, activation, antigen processing or presentation, these DC surprisingly presented an enhanced ability to activate T cells ex vivo and in vivo. These data demonstrate that leptin can modulate DC function and suggest that leptin may dampen T-cell responsiveness in the physiological setting.

Keywords: CD4<sup>+</sup> T cells, cytokines, helper T cells, T-cell activation

Introduction

Leptin, the product of the obese (ob) gene, is a 16-kDa protein produced primarily by mature white adipose tissue (1), with leptin serum levels being proportionate to adiposity (2). Research has shown that leptin plays an important role in food intake and energy expenditure (3), in addition to its roles in other physiological processes such as reproduction, glucose and insulin metabolism, and hematopoiesis. Leptin exerts its effects by interacting with its cognate receptor, which is encoded by the diabetes (db) gene (4, 5). Alternative mRNA splicing gives rise to six variants of the leptin receptor (ObR), of which only the long isoform has been reported to be crucial for leptin signal transduction through the JAK/STAT pathway (5–8). The long isoform of the leptin receptor is highly expressed in the arcuate nucleus of the hypothalamus, and to a much lesser degree in the lungs and kidneys and has been detected in other organs and cells types including T cells, B cells, monocytes and macrophages (9, 10).

Leptin has been shown to affect both the innate and adaptive branches of the immune system. For innate immunity, leptin modulates activity of NK cells (11), macrophages (12–14) and neutrophils (15), potentiating function in all cell types and promoting the production of proinflammatory cytokines. For adaptive immunity, in vitro and in vivo experiments demonstrate that leptin positively influences T-cell proliferation and increases T<sub>1</sub> cytokine production while suppressing T<sub>2</sub> (13, 16–18). These findings are further substantiated by bacterial infection and experimental autoimmune disease models. Leptin-deficient (Lep<sup>ob</sup>) or leptin receptor-deficient (Lep<sup>db</sup>) animals have impaired ability to clear or control infection by Klebsiella pneumoniae (19), Listeria monocytogenes (20), and Mycobacterium tuberculosis (21) and are less susceptible to experimental autoimmune encephalomyelitis (EAE) (22) and experimental arthritis (23). The leptin-deficient animals were characterized with low leukotriene synthesis (19) and a T<sub>1</sub> phenotype (22, 23). Similar experiments where leptin

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is administered exogenously have been shown to accelerate EAE (24) and autoimmune diabetes in non-obese diabetic mice (25), both models present with an increase in the T<sub>1</sub> pattern of cytokine release. Taken together, the data support a model in which leptin exerts its effects on the immune system by promoting proinflammatory responses.

Integration of the innate and adaptive immune responses is mediated by dendritic cells (DC), which are the only reported cells capable of activating naive T cells (26–28). Leptin has also been shown to modulate DC: the addition of exogenous leptin to human monocyte-derived DC resulted in enhanced DC survival, induction of a T<sub>1</sub>1 response as measured by cytokine production by the treated DC and the responding T cells, and re-arrangement of actin cytoskeleton, resulting in enhanced migratory capabilities (11, 29). DC derived from the bone marrow (BM) of Lep<sup>−/−</sup> or Lep<sup>0/0</sup> mice showed the corresponding opposite results: poor survival, a T<sub>2</sub> or TGF-β cytokine profile, and a poor capacity to stimulate allogeneic T cells (30, 31). Thus, leptin appears to also be a critical for optimal DC function.

Taken together, these data suggest that leptin is required for optimal cell-mediated immunity. Leptin potentiates innate immune cell activity (13, 14, 26, 32), including that of DC (11, 29–31), and enhances T-cell responsiveness (11, 16–18, 33). Specifically, leptin promotes survival and migration of DC and induces T<sub>1</sub>-mediated inflammation while seemingly suppressing T<sub>2</sub>-mediated responses. However, these findings are based on DC generated from stem cells of humans and mice and are based on the acute exposure of DC to exogenous leptin. Data focused on the effects on DC in situ are scant; one study found that leptin deficiency increased the steady-state number of DC in the epidermis (31); the functionality of this specific DC population, however, was not ascertained.

Given the importance of DC in the initiation and regulation of an immune response, the present study was designed to evaluate the effect of leptin on DC function in vivo, focusing on a population of DC that have differentiated in the absence of leptin. The study assessed the effect of leptin deficiency on splenic DC (sDC). Our findings demonstrate that, while leptin is not crucial for the ability of sDC to respond to inflammatory stimuli nor to acquire or process antigen, the absence of leptin enhances their ability to activate allogeneic and antigen-specific T cells ex vivo. Moreover, leptin deficiency allowed for increased clearance of systemically administered bacteria that correlated with an increased percentage of activated T cells. The data demonstrate that for this specific population of DC, in vivo chronic exposure to normal circulating levels (lean levels) of leptin negatively modulates DC function. The data suggest that the physiological purpose of leptin, at normal concentrations, is to dampen DC activation of antigen-specific T cells. Moreover, the data underscore that stem cell-derived DC acutely exposed to high levels of leptin are not necessarily representative of how leptin affects DC function.

**Methods**

**Animals**

Three weeks old female Lep<sup>−/−</sup> and their heterologous control littermates (C57Bl/6) were purchased from Jackson Laboratories (Bar Harbor, ME, USA) and used for experiments at 8 weeks. The obese mutation, ob, arose spontaneously in strain V/Le at The Jackson Laboratory in 1949 and has been back-crossed to C57BL/6J for >45 generations. The mutation causes production of non-functional leptin. Six weeks old female Lep<sup>−/−</sup> mice and Balb/c female mice, 6–8 weeks old, were also purchased from Jackson Laboratories. The spontaneous autosomal recessive mutation diabetes, db was discovered at The Jackson Laboratory in 1966 on the inbred strain C57BLKS/J. Formerly known as db, after cloning it became Lep<sup>−/−</sup>. The mutation has been back-crossed to C57Bl/6J mice for 85 generations and causes production of a non-functional leptin receptor. Animals were kept in a controlled environment with a constant temperature of 72° Fahrenheit and a 12:12 light–dark cycle. Animals were fed standard pellet chow ad lib. All experiments were conducted in accordance with Institutional Animal Care and Use Committee and University of Texas at El Paso guidelines.

**sDC isolation**

To isolate sDC, spleens were treated with collagenase D (Sigma-Aldrich; 1 mg ml<sup>−1</sup>) for 15 min, teased apart and then treated a second time with collagenase D for 30 min. The disintegrating spleens were further separated with a sterile syringe plunger and were then filtered to remove cellular debris. Following a wash in HBSS (Invitrogen), sDC were purified using magnetic-activated cell sorting (MACS) (Miltenyi Biotech) via positive selection, as described below.

**T-cell hybridoma**

C57Bl/6-derived ovalbumin-specific CD4<sup>+</sup> T cell hybridoma (80.10) cells were a generous gift from Dr Phillipa Marrack (National Jewish Medical and Research Center). Hybridoma were kept in culture in hybridoma media (S-MEM supplemented with 30% tumor cocktail) and were used every fourth day after passaging. Tumor cocktail includes d-glucose, glutamine, non-essential amino acids, sodium pyruvate, sodium bicarbonate, gentamycin, penicillin G, streptomycin sulfate and 2-mercaptoethanol.

**MACS**

Single cell suspensions of spleens were incubated in 30% mouse serum (Rockland) in MACS buffer (2% fetal bovine serum in 1×PBS) (Hyclone). Antibody-conjugated magnetic beads (Miltenyi) against the DC marker CD11c were added at a ratio of 90 μl for every 10<sup>6</sup> cells. Following incubation on ice, the cells were placed onto a positive selection magnetic column (Miltenyi) and washed three times with MACS buffer. Upon removal from the magnet, the enriched cell suspension was released from the column using pressure. Cell purity, as determined by flow cytometry, was consistently above 95%.

**Measurement of Ob-R and STAT-3 activation**

sDC expression of the leptin receptor as well as receptor functionality, were determined by flow cytometry. Briefly, sDC single cell suspensions were generated; half the cells were treated with lysis buffer to remove RBCs and half were enriched for DC by MACS purification. The first half were
stained with anti-CD11c (BD Pharmingen) to identify the percentage of DC in the spleen and with anti-murine leptin receptor (R&D Systems) to determine Ob-R expression. After fixation with 1% paraformaldehyde (PFA) (Sigma Aldrich) the cells were analyzed by flow cytometry. To determine the receptor’s ability to activate the JAK2/STAT3 pathway, sDC were analyzed for pY705. Briefly, sDC were isolated and treated with 10nM leptin. At the indicated times, the cells were harvested, fixed with 1% PFA, permeabilized with Perm Buffer (BD Pharmingen) and were then stained with anti-pyS-TAT3 (BD Pharmingen). The cells were then later analyzed by flow cytometry.

DC phenotype
To assess phenotype, enriched sDC from experimental or control mice were blocked with 30% normal mouse serum (NMS), incubated with PE-conjugated antibodies (BD Pharmingen) against the cell surface markers CD80, 86, 54 and 40 (each diluted 1:50). In each case, the cells were fixed with 1% PFA and analyzed by flow cytometry.

Flow cytometry
Specificity of staining was controlled with isotype-matched antibodies for all studies (BD Pharmingen). Flow cytometry analysis was performed on a Beckman-Coulter FC500 instrument. Samples were gated for live cells based on forward- and side-scatter parameters and 10000 events per sample were collected and analyzed using CXP software (Beckman-Coulter).

DC activation
To assess the response of enriched sDC to inflammatory stimuli, DC cultures were treated with LPS (Sigma-Aldrich; 10 μg ml⁻¹) or were incubated with hamster anti-mouse-CD40 (HM40-3, 5 μg ml⁻¹) and goat anti-hamster immunoglobulins (BD Pharmingen). Following the activation period, the cells were blocked with 30% NMS and labeled with PE-conjugated antibodies to CD80, 86, 54 and 40. The cells were then fixed with 1% PFA and were analyzed by flow cytometry.

DC phagocytic activity
Enriched sDC from Lep⁻ and control mice were used to assess phagocytic activity. Phagocytosis was monitored using the Vybrant Phagocytosis Assay Kit (Molecular Probes) as per the manufacturers’ instructions. Briefly, cells were seeded at 1 x 10⁶ cells per well in a 96-well flat bottom, black-walled plate and incubated with fluorescein-labeled Escherichia coli fragments. Following incubation, the cells were treated with trypan blue dye to quench fluorescence of any bacterial fragments that were not taken up by the DC, i.e. remained extracellular. Phagocytosis was analyzed with a fluorescence plate reader. Alternatively, phagocytosis was measured by uptake of the GFP-expressing Mycobacterium avium strain JC104 (a generous gift from Dr Todd P. Primm, Sam Houston State University). The cells were plated in 12-well plates at 1 x 10⁶ cells per well and cocultured with the bacterial cells at a multiplicity of infection of 5 bacteria per DC. After incubation, the DC were washed with amikacin (Sigma-Aldrich; 200 μM) to remove extracellular M. avium. The infected cells were stained with anti-CD11c to identify DC and assessed for bacterial internalization as a function of relative GFP fluorescence by flow cytometry.

DC antigen processing
Enriched sDC from Lep⁻ and control mice were cultured in 96-well, flat bottom, black-walled plates in the presence of caged fluorescently labeled ovalbumin (OVA-DQ) (Molecular Probes; 10 μg ml⁻¹). OVA-DQ fluorescence was detected with a fluorescence plate reader. Fluorescence was excited at λ505 and detected at λ515 at 10-min intervals over a 320 min time course.

Mixed lymphocyte reactions
Enriched sDC from Lep⁻ or control mice were used as stimulators of CD4⁺ T cells enriched from lymph nodes of Balb/c mice. sDC were isolated as described above, irradiated in an XRad160 γ-iradiator (Precision Instruments; 1200 rads), then cocultured with responder T cells at a 1:5 ratio. T-cell activation was measured 96 h later as a function of IFN-γ production (measured by ELISA) or of proliferation. To assess proliferation, cells were labeled with [³H]-thymidine (PerkinElmer; 0.5 μCi) during the final 18 h of culture (overnight pulse). The cells were harvested and the cell-associated radioactivity was determined by a β-counter. Un-enriched lymph node cells stimulated with Con A were used as a positive control and T cells in culture medium were used as a negative control.

Antigen-specific presentation
Enriched sDC from leptin-deficient and control mice were pulsed with increasing concentrations of I-A⁻ restricted ovalbumin peptide 323–339 (H-Ile-Ser-Gln-Ala-Val-His-Ala-Asn-Glu-Ala-Gly-Arg-OH) (Peptides International) and cocultured with ovalbumin peptide specific CD4⁺ T-cell hybridoma cells (80.10) at a ratio of 1:5, DC to T cells. T-cell hybridoma activation was detected as a function of IL-2 production by ELISA after a 96-h coculture.

ELISA
Cellular production of IFN-γ and IL-2 was measured by ELISA. Microwell ELISA plates were coated with capture antibody. Plates were blocked at room temperature with 3% BSA in PBS. Supernatants from stimulated T cell and sDC cocultures were added to the plates. Following binding of cytokines to the capture antibodies, the plates were incubated with biotin-conjugated anti-cytokine antibody, followed by HRP-labeled avidin (Biosource). The enzyme substrate O-phenylenediamine (Sigma-Aldrich) was utilized for color development. Cytokine concentrations were calculated against murine recombinant cytokines (BD Pharmingen).

In vivo infection of mice with M. avium
Following anesthetization, the mice were injected intraperitoneally with 1 x 10⁶ cells of M. avium strain MAC104 (obtained from The Institute for Genomic Research). At
the indicated time post-infection, spleen and lungs were collected in HBSS and placed with 1 mm glass beads in a bead beater (Biospec Products Inc.); cells were disrupted for three continuous minutes. Serial dilutions (10^{-4}, 10^{-5} and 10^{-6}) of the tissue homogenates were plated onto Mycobacteria 7H11 agar (VWR) supplemented with Oleic Acid Dextrose Complex (OADC) (100 ml l^{-1}) (VWR). Colonies were counted on day 14 and bacterial titers were calculated by multiplying the number of colonies by the dilution factor to determine the number of colony-forming units, generating an average over the different dilutions plated.

Statistical analysis
Where applicable, data were analyzed by the appropriate statistical test (Student’s t-test and one-way ANOVA) to determine significant differences. Statistical analyses were conducted in consultation with the Border Biomedical Research Center Statistical Consulting Lab.

Results
Murine sDC express a functional leptin receptor
To determine whether the absence of leptin alters DC function, the current studies were focused on sDC, working from the assumption that this population had developed, differentiated and seeded peripheral tissue entirely within the leptin-deficient environment. This is in contrast to BM-DC, which are exposed to varying levels of bovine leptin in the FCS used to supplement the media and, by nature of the culture, do not seed peripheral tissue. sDC were therefore assessed for the presence of the leptin receptor by flow cytometry. As shown in Fig. 1(A), ~6% of C57Bl/6 and Lep^{-ob} splenocytes are DC (CD11c^{+}); moreover, the percentage of sDC expressing the leptin receptor was equivalent for both genotypes as was the relative expression level of ObR (Fig. 1A). The antibody used in these studies, however, identifies all isoforms of the leptin receptor since it is specific for the extracellular domain.

To determine the functionality of the leptin receptors present, leptin-treated sDC were analyzed by flow cytometry.

Fig. 1. Murine sDC express functional leptin receptor. (A) Single cell suspensions of C57Bl/6 and Lep^{-ob} (leptin deficient) spleens were analyzed by flow cytometry to determine the percentage of splenocytes that were DCs (sDC). The splenocytes were then gated for DC (CD11c) and analyzed for the cell surface expression of Ob-R. (B) Single cell suspensions of C57Bl/6 and Lep^{-db} (leptin receptor-deficient) spleens were treated with 160 ng ml^{-1} of leptin; at the indicated time points, the cells were harvested, stained with anti-CD11c, fixed, permeabilized, stained with anti-py705 STAT-3 and then analyzed by flow cytometry. The splenocytes were gated for DC (CD11c) and analyzed for the percentage of cells expressing phosphorylated STAT-3. All data are one of three representative experiments and are presented as the mean ± SEM of duplicate samples. * P < 0.005 by Student’s t-test.
for the presence of phosphorylated STAT-3. sDC from leptin receptor-deficient mice (Lep<sup>ob</sup>) were used as controls. As presented in Fig. 1(B), the percentage of sDC with phosphorylated STAT-3 was higher in C57Bl/6 mice than in Lep<sup>ob</sup> mice at 15 and 30 min following leptin treatment. The data clearly demonstrate that treatment of sDC with leptin induces signaling through the JAK/STAT signaling pathway suggesting that sDC express the signaling (long) isoform of the leptin receptor.

sDC percentages and phenotypes are similar between C57Bl/6 and Lep<sup>ob</sup> mice

Evaluation of the impact of leptin deficiency on DC began with an overall assessment of the sDC population. The spleens of C57Bl/6 and Lep<sup>ob</sup> mice were analyzed for total DC percentages and the sDC were analyzed for phenotype. The percentage of CD11c and class II MHC<sup>+</sup> cells was determined by flow cytometry. No detectable differences were observed in sDC percentages between the two genotypes (Fig. 1A). The CD11c and class II MHC<sup>+</sup> cells were further analyzed for the relative expression levels of antigen-presenting cell (APC) surface markers CD80, CD86, CD54 and CD40. Again, no detectable differences were observed between control and experimental sDC populations (Fig. 2A vs. C or B vs. D, focusing on the solid black bars, which equals unstimulated sDC). These results show that the overall number and sDC phenotype remain unaffected by the absence of leptin.

C57Bl/6 and Lep<sup>ob</sup> sDC respond similarly to external stimuli

Given the role of DC as APC and activators of naive T cells, it was critical to assess whether the absence of leptin would alter sDC function, beginning with the ability to respond to inflammatory stimuli, which is required for optimal interaction and activation of T cells. To determine this, enriched sDC were exposed to LPS to mimic bacterial stimulation, or to CD40 cross-linking to simulate activation upon interaction with T cells. Following LPS or anti-CD40 treatment, the sDC were stained for the presence of APC activation markers and were analyzed by flow cytometry (Fig. 2). The results demonstrate that there were no detectable differences, since the extent of stimulation (upregulation of APC activation markers) was similar between the two genotypes regardless of the stimulus. The results imply that sDC, which differentiate in a leptin-deficient environment, retain their ability to respond to external stimuli.

![Fig. 2](https://academic.oup.com/immunology/article-abstract/26/11/627/2950791)
Lep\(^{-}\) sDC possess normal antigen acquisition and processing capabilities

To determine whether leptin deficiency compromises the ability of sDC to acquire antigen, enriched populations of Lep\(^{-}\) and C57Bl/6 sDC were fed bacteria that were either fluorescently labeled or that expressed GFP. Enriched sDC were first exposed to fluorescently labeled E. coli fragments. Immediately after incubation, the bacterial media was removed and external fluorescence was quenched by treating the cells with trypan blue. Phagocytosis was then measured as a function of fluorescence intensity. The results showed no significant differences in phagocytosis of the E. coli fragments between Lep\(^{-}\) and control sDC (Fig. 3A). To determine whether this observation was pathogen-specific, ingestion of GFP-expressing M. avium was also assayed. To remove extracellular bacteria, the cells were washed with amikacin following the incubation period. The sDC were fixed and analyzed by flow cytometry to identify CD11c\(^{+}\) cells that were also GFP\(^{+}\). The results showed that there were no significant differences in phagocytosis of the GFP-expressing M. avium by the Lep\(^{-}\) sDC when compared with the controls (Fig. 3B). However, acquisition of protein antigens is meaningless if the APC is unable to convert the proteins to peptides for antigen presentation. Thus, antigen processing was also assessed. Enriched sDC from Lep\(^{-}\) and C57Bl/6 mice were incubated in the presence of OVA-DQ, which is caged fluorescein-labeled ovalbumin. Antigen processing was determined as a function of fluorescence intensity released upon proteolytic removal of the cage. As shown in Fig. 3(C), Lep\(^{-}\) sDC reveal similar antigen-processing capabilities relative to controls. Thus, neither antigen acquisition nor antigen processing is compromised in sDC that have differentiated in a leptin-deficient environment.

Lep\(^{-}\) sDC induce stronger T-cell responses in mixed lymphocyte reactions and antigen-specific assays

The final and ultimate function of DC is to present antigen to T cells for the initiation of adaptive immunity. To evaluate whether leptin deficiency alters the ability of sDC to activate T cells, mixed lymphocyte reactions (MLRs) and antigen-specific T-cell activation assays using a T-cell hybridoma cell line were conducted. Enriched and irradiated sDC from Lep\(^{-}\) or C57Bl/6 mice were used as stimulators of CD4\(^{+}\) primary T cells enriched from lymph nodes of Balb/c mice. T-cell stimulation was measured after 72h of coculture as a function of IFN-γ production and the comparative rate of tritiated-thymidine incorporation after 96h of coculture. As shown in Fig. 4(A and B), Lep\(^{-}\) sDC elicited a higher response than the control.

Fig. 3. Lep\(^{-}\) sDC possess normal antigen acquisition and processing capabilities. (A) Enriched Lep\(^{-}\) and control sDC were cocultured with FITC-labeled E. coli fragments. The cells were treated with trypan blue to quench extracellular fluorescence and were analyzed by flow cytometry to assess phagocytosis as a function of fluorescence intensity. Control = background (cells alone). (B) Enriched Lep\(^{-}\) and control sDC were cocultured with GFP-expressing M. avium (multiplicity of infection = 5). The cells were washed with 200 μM amikacin to remove extracellular bacteria. The cells were stained with anti-MHC class II and anti-CD11c to identify DC and were assessed for bacterial internalization as a function of relative GFP fluorescence. (C) Lep\(^{-}\) and control DC were enriched and pulsed with caged fluorescently labeled ovalbumin (OVA-DQ) and were monitored for fluorescence emission every 10min for 320min. All data are presented as the mean ± SEM for duplicate samples for each animal and are one of two representative experiments.
were repeated using sDC isolated from leptin receptor-deficient mice (Lepob). As shown in Fig. 4(E), Lepob sDC also induced enhanced activation of the ovalbumin-specific T-cell hybridoma. These data demonstrate that the level of bovine leptin in the FCS is insufficient to induce the observed T-cell activation phenotype since leptin receptor-deficient DC are able to induce the same level of activation. Moreover, these sets of data also demonstrate that leptin deficiency in vivo does not alter the leptin-signaling sensitivity of sDC since the absence of the leptin receptor yields sDC with equivalent functionality. Thus, leptin deficiency results in sDC with more potent stimulatory ability ex vivo suggesting that, in vivo, leptin may be exerting inhibitory effects on sDC as pertains to their ability to activate T cells.

Lepob mice are more efficient at neutralizing M. avium infections when compared with wild-type controls in vivo

To determine whether the findings from the MLRs and antigen-specific assays were not the result of an in vitro artifact,

Fig. 4. sDC from Lepob mice are strong activators of primary or antigen-specific T cells. Enriched and irradiated sDC from C57Bl/6 and Lepob mice were used as stimulators for an MLR using CD4+ T cells enriched from lymph nodes of Balb/c mice as the responders. T-cell stimulation was measured at 72h as a function of IFN-γ production by ELISA (A) and at 96h for thymidine incorporation (B). Enriched sDC from C57Bl/6 and Lepob mice were pulsed with increasing concentrations of ovalbumin peptide and used as antigen presenters to a C57B/6-derived ovalbumin-specific CD4+ T-cell hybridoma (80.10). T-cell responses were measured as a function of IL-2 production (C). Single cell suspensions of spleens from C57Bl/6 and Lepob mice were stained with anti-CD11c and anti-ObR and assessed by flow cytometry to determine the relative expression level of the leptin receptor on the DC (D). Enriched sDC from C57Bl/6 and Lepob mice were pulsed with 313 μg ml−1 of ovalbumin peptide and used as antigen presenters to T-cell hybridoma (80.10). T-cell responses were measured as a function of IL-2 production (E). Data are presented as the mean ± SEM of triplicate samples and are one of three representative experiments. ND = none detected. Statistical analysis: *(A, B and E) P ≤ 0.05 by Student’s t-test. *(C) P ≤ 0.0001 and **(C) P ≤ 0.001 by one-way ANOVA with Bonferroni post-test.
mice were infected with *M. avium*. C57Bl/6 and Lep°/° mice were intra-peritoneally infected with 1 × 10⁸ bacteria to mimic a systemic infection. Every 15 days post-infection and up to 60 days, the bacterial load in the spleen and lungs was determined to assess the extent of infection. As seen for the *in vitro* results, leptin-deficient mice were significantly better at controlling *M. avium* than the control mice were as evidenced by better containment of overall bacterial loads in the spleen (Fig. 5A). Lung infection was undetected in both genotypes. Moreover, splenic bacterial loads directly correlated with the percentage of activated CD4⁺ splenic T cells (Fig. 5B), suggesting that in Lep°/° mice the bacteria were managed by a slightly larger and perhaps more effective T-cell population. These results further substantiate the *in vitro* findings and demonstrate that leptin deficiency enhances sDC-mediated T-cell activation.

Discussion

Circulating concentrations of leptin are proportional to adiposity, making leptin a biomarker for body fat and a reflection of individual energy balance (3). The primary function of leptin is in the regulation of appetite and energy expenditure, but studies over the years have provided evidence to suggest that it is a pleiotropic adipokine that influences reproduction, glucose metabolism, angiogenesis, production of surfactant and immunity (4, 5). Data have demonstrated that human and murine DC express the signaling isofrom of the leptin receptor and that addition of exogenous leptin promotes DC survival, alters DC morphology leading to increased migration and pushes DC to induce a T₅₁ cytokine profile (11, 29–31). While interesting, these studies used DC that were differentiated *ex vivo* and may have been functionally influenced by *in vitro* factors. Thus, given the important role of DC in immunity and the reported enhancement of DC function induced by leptin, the current investigation was designed to determine the impact of leptin deficiency on a population of DC that had differentiated and matured entirely within a leptin-deficient environment.

To assess the influence of leptin deficiency on sDC function we first ascertained whether sDC express the signaling isofrom of the leptin receptor, a requirement for leptin to exert its effects (Fig. 1). Our findings show that sDC express the leptin receptor and that the receptor is functional, suggesting that sDC are capable of responding to circulating leptin. We continued with an assessment of sDC percentages and phenotype, both of which were equivalent between cellular suspensions from leptin-deficient and C57Bl/6 control spleens (Figs 1 and 2). These data suggest that the DC percentages required to mount an immune

![Fig. 5. Leptin-deficient mice neutralize *M. avium* infections more efficiently than control mice. Lep°/° and control mice were infected with 1 × 10⁸ *M. avium* cells via an intra-peritoneal administration. On the indicated days post-infection, splenic bacterial loads were quantified (A) and the percentage of activated T cells (CD69-positive) in the spleen (B) were determined for CD4⁺ cells by flow cytometry. For A, data are presented as the mean ± SEM of three different animals; for B, data are presented as the mean ± SEM of triplicate samples. The data are one of three representative experiments. *(A) P ≤ 0.0001 by Student’s *t*-test. *(B) P ≤ 0.002 by Student’s *t*-test.](https://academic.oup.com/intimm/article-abstract/26/11/627/2950791)
response are not altered by the absence of leptin nor is the ability of the sDC to interact with naive T cells since cell surface markers remained present on the sDC despite the lack of leptin. Our data however, are in contrast to findings made by Macia et al. (31), which demonstrate that leptin-deficient mice present with more DC in the spleen than their normal counterparts. Similar findings were made for the epidermis of these mice where higher numbers of epidermal DC were found in leptin-deficient mice compared with control animals. Spontaneous migration of epidermal DC from skin explants of leptin-deficient mice appeared normal, as did inducible migration in vivo (31). In our studies, the sDC from leptin-deficient mice responded normally to inflammatory stimuli (Fig. 2) and were able to acquire and process antigen similar to control sDC (Fig. 3). Thus, despite differing in cell numbers, the two studies demonstrate that peripheral DC are functionally normal. It is possible that seeding of peripheral tissues by DC varied between the two studies as a result of genetic differences, since the leptin-deficient mice used by Macia et al. possess only 70% of the C57Bl/6J background, whereas ours possess an entirely C57Bl/6 genetic background. In addition, Asselin-Paturel et al. (34) reported that plasmacytoid DC frequency and function differ among different mouse strains, thus further supporting the notion that DC cell numbers and function differ from strain to strain.

Antigen presentation to naive T cells in the draining lymph nodes of peripheral tissue is one of the characteristics that set DC apart from other APC. DC are critical for the initiation and regulation of an immune response and must therefore be evaluated for their ability to induce T-cell immunity. BM-derived DC from leptin-deficient mice have been shown to be less immunogenic than controls (31); we therefore hypothesized that sDC from Lep<sup>ob</sup> mice would likely be suboptimal APC. Despite appearing functionally normal in regard to activation, antigen acquisition and antigen processing, sDC from leptin-deficient mice were surprisingly different than controls in their ability to activate T cells. Our results demonstrate that Lep<sup>ob</sup> sDC were more effective activators of T cells in an MLR and in an antigen-specific T-cell hybridoma assay (Fig. 4). These data were further supported by our findings, which show that DC isolated from leptin-receptor-deficient mice were also more efficient at activating primary antigen-specific T cells (Fig. 4E). Together, the data demonstrate that the absence of leptin signaling in vivo promotes the maturation of a more immunogenic DC, which is independent of a change in leptin-signaling sensitivity since sDC from Lep<sup>ob</sup> mice lack the ability to signal. Differentiating and maturing completely within the leptin-deficient environment seems to have produced a distinctly unique population of DC, allowing for enhanced immunogenicity. Our in vivo data substantiated the ex vivo data in that clearance of a systemic bacterial infection was superior in the leptin-deficient environment and was accompanied with an increase in the percentage of activated T cells (Fig. 5). These data also support the premise that the observed phenotype is not due to changes in leptin-signaling sensitivity since exogenous leptin is not added to the in vivo studies.

Earlier findings have demonstrated that Lep<sup>ob</sup> or Lep<sup>ob</sup> mice are more susceptible to bacterial infections and less susceptible to the induction of experimental autoimmune disease (19–23), due in large part to the need for leptin by the responding T cells. In fact, the administration of leptin reverses T-cell responses to normal. Our data, however, demonstrate enhanced T-cell responsiveness in Lep<sup>ob</sup> mice, which are in line with published reports demonstrating that ob mice present reduced disease severity in pre-clinical models of intestinal inflammation induced by dextran sulfate sodium and autoimmune hepatitis induced by Con-A (35, 36). This may be due to differences in the route of administration, therefore targeting a different population of DC. Thus, it might be the specific population of DC and their microenvironment that affects whether or not they are influenced by leptin and to what extent. Indeed, intestinal DC have been reported to be functionally modulated by products of adjacent intestinal epithelial cells (37) and BM-DC have been shown to become tolerogenic when exposed to products of thymic stromal cells (38). Both reports provide evidence that the local microenvironment can impact DC function, which may explain the unique functional ability of sDC isolated from Lep<sup>ob</sup> mice.

In summary, we have evaluated a specific population of DC to determine the effect of leptin deficiency on DC function. We report that, for sDC, the absence of leptin enhances DC induction of T-cell responsiveness ex vivo and in vivo. The data therefore imply that in the physiological setting, sustained exposure to normal levels of leptin may function to temper T-cell responses induced by sDC. This may be of particular importance when considering potential anti-leptin therapies proposed for treatment of excess inflammation and increased susceptibility to infections. Excess leptin, commonly caused by obesity, has been shown to reduce T<sub>1</sub> responses, to alter inflammatory responses and to result in an increased susceptibility to pulmonary and urinary tract infections (39). Excessive reduction of leptin by anti-leptin therapies to counteract these adverse symptoms could potentially bring on a new series of complications, such as increased immunogenicity, as highlighted by the data presented in this report. It is therefore critical to evaluate the effects leptin, and any pleiotropic molecule, on all cells of the immune system and on subpopulations within individual cellular compartments to strike an appropriate balance of immune responsiveness.

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