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J Immunol (2002) 168 (8): 4018–4024.

<https://doi.org/10.4049/jimmunol.168.8.4018>

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Leptin-Deficient Mice Exhibit Impaired Host Defense in Gram-Negative Pneumonia¹

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Leptin is an adipocyte-derived hormone that is secreted in correlation with total body lipid stores. Serum leptin levels are lowered by the loss of body fat mass that would accompany starvation and malnutrition. Recently, leptin has been shown to modulate innate immune responses such as macrophage phagocytosis and cytokine synthesis *in vitro*. To determine whether leptin plays a role in the innate host response against Gram-negative pneumonia *in vivo*, we compared the responses of leptin-deficient and wild-type mice following an intratracheal challenge of *Klebsiella pneumoniae*. Following *K. pneumoniae* administration, we observed increased leptin levels in serum, bronchoalveolar lavage fluid, and whole lung homogenates. In a survival study, leptin-deficient mice, as compared with wild-type mice, exhibited increased mortality following *K. pneumoniae* administration. The increased susceptibility to *K. pneumoniae* in the leptin-deficient mice was associated with reduced bacterial clearance and defective alveolar macrophage phagocytosis *in vitro*. The exogenous addition of very high levels of leptin (500 ng/ml) restored the defect in alveolar macrophage phagocytosis of *K. pneumoniae* *in vitro*. While there were no differences between wild-type and leptin-deficient mice in lung homogenate cytokines TNF- α , IL-12, or macrophage-inflammatory protein-2 after *K. pneumoniae* administration, leukotriene synthesis in lung macrophages from leptin-deficient mice was reduced. Leukotriene production was restored by the addition of exogenous leptin (500 ng/ml) to macrophages *in vitro*. This study demonstrates for the first time that leptin-deficient mice display impaired host defense in bacterial pneumonia that may be due to a defect in alveolar macrophage phagocytosis and leukotriene synthesis. *The Journal of Immunology*, 2002, 168: 4018–4024.

Leptin, the product of the *ob* gene, is secreted primarily by adipocytes in correlation with total body fat mass and is rapidly lowered by fasting (1, 2). This 16-kDa protein plays an important role in the regulation of energy balance (3). In the C57BL/6J *ob/ob* mouse, a mutation in the *ob* gene results in the formation of a nonfunctional protein and leptin deficiency (1, 4, 5). Leptin deficiency in the *ob/ob* mouse or during starvation in a normal animal leads to hunger, decreased energy expenditure, and a series of hormonal and immune abnormalities. The restoration of circulating leptin levels reverses these abnormalities (6–9). While human *ob* gene mutations are rare, food restriction and starvation represent common situations in which serum leptin levels would be decreased in humans with chronic illnesses such as cancer, cystic fibrosis, and emphysema (2, 10–13). Malnutrition is an important cause of immune suppression and increases the host's susceptibility to infectious diseases such as pneumonia (14, 15), the most common cause of death due to infectious disease in the United States (16). While malnutrition is known to suppress immune function, the mechanisms responsible for compromised antimicrobial host defense are not well understood.

Recently, several reports have identified a role for leptin in regulating immune function (17–19). Macrophages harvested from leptin-deficient mice exhibit attenuated phagocytosis *in vitro*, and the exogenous addition of leptin to normal macrophages has been shown to augment macrophage phagocytosis and killing of bacteria and cytokine synthesis *in vitro* (17, 19). Interestingly, infection, sepsis, LPS, TNF- α , IL-1, and leukemia inhibitor factor have been shown to increase serum leptin levels *in vivo* (8, 9, 20, 21). This suggests that leptin participates in the acute phase response to inflammation and may play a role in the host response to infection.

In this study, we asked whether leptin played an important role in the host defense against Gram-negative pneumonia by comparing the responses of wild-type (WT)³ and leptin-deficient mice to an intratracheal challenge with *Klebsiella pneumoniae* *in vivo* and macrophage functions *in vitro*. We observed that leptin-deficient mice, as compared with WT mice, exhibited greater mortality and reduced bacterial clearance from the lung following an intratracheal challenge of *K. pneumoniae*. The increased susceptibility to bacterial pneumonia in the leptin-deficient mice was associated with reduced alveolar macrophage phagocytosis of *K. pneumoniae* *in vitro* that could be restored with exogenously added leptin.

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Received for publication August 20, 2001. Accepted for publication February 15, 2002.

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¹ This work was supported by grants from the American Lung Association (RG-056-N) and the University of Michigan Office of the Vice President of Research.

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Materials and Methods

Animals

Female C57BL/6j-*ob/ob* and C57BL/6j WT mice were obtained from The Jackson Laboratory (Bar Harbor, ME) and maintained under specific pathogen-free conditions. All experiments were conducted in compliance with the Animal Care and Use Committee of the University of Michigan.

³ Abbreviations used in this paper: WT, wild type; BALF, bronchoalveolar lavage fluid; LTB₄, leukotriene B₄; MIP-2, macrophage-inflammatory protein-2.

K. pneumoniae inoculation

K. pneumoniae strain 43816, serotype 2, was obtained from the American Type Culture Collection (Manassas, VA), and aliquots were grown in tryptic soy broth (Difco, Detroit, MI) for 18 h at 37°C. The concentration of bacteria in culture was determined spectrophotometrically (A_{600}) (22). The bacteria were serially diluted in endotoxin-free saline to obtain the appropriate concentration. Following anesthesia with ketamine and xylazine (100 and 10 mg/kg body weight, respectively), a midline incision was made to expose the trachea, a 30- μ l inoculum containing 5000 CFU *K. pneumoniae* or saline was administered via the trachea using a 30-gauge needle, and the wound was closed using surgical glue (Nexaband, Phoenix, AZ).

Determination of blood and lung CFU

Blood and lung homogenate CFU were determined as previously described (22). Briefly, lungs were homogenized in 1 ml sterile saline and blood was collected from euthanized mice 2 days post-*K. pneumoniae* challenge. Serial dilutions of each sample were plated on soy-based blood agar plates (Difco). After 18 h at room temperature, CFU were enumerated.

Cytokine and leptin determinations

Cytokines and leptin were extracted from murine lungs, as previously described (23). In short, lungs were perfused with saline, removed from mice en bloc, and homogenized in buffer containing 0.5% Triton X-100, 150 nM NaCl, 15 nM Tris-HCl, 1 mM CaCl₂, and 1 mM MgCl₂. Lung homogenates were centrifuged at 5000 rpm for 10 min, and the supernatants were filtered through a 0.45- μ m filter and stored at -70°C. Murine TNF- α and IL-12 in lung homogenates were determined using commercially available ELISA kits (OPTeia kits; BD PharMingen, San Diego, CA). Murine macrophage-inflammatory protein-2 (MIP-2) was determined using a modification of a double-ligand ELISA method, as previously described (24). Murine leptin in lung homogenates, blood, and bronchoalveolar lavage fluid (BALF) was determined using a commercially available enzyme immunoassay kit obtained from Diagnostic Systems Laboratories (Webster, TX).

Lung leukocyte preparation and assessment

Lungs were excised from each mouse, washed with PBS, minced with scissors, and digested enzymatically for 30 min in 15 ml digestion buffer (RPMI 1640, 5% FCS, 1 mg/ml collagenase (Boehringer Mannheim, Chicago, IL), and 30 μ g/ml DNase (Sigma-Aldrich, St. Louis, MO)), as previously described (25). A purified population of leukocytes was obtained after subsequent sample processing involving tissue fragmentation, erythrocyte lysis, filtration, and density gradient centrifugation to remove cell debris and epithelial cells. The total number of viable lung leukocytes was determined by trypan blue exclusion using a Neubauer hemocytometer. Differential counts were determined after cells had been cytospun onto glass slides using a Shandon Cytospin 2 (Thermo Shandon, Pittsburgh, PA) and stained using a modified Wright-Giemsa stain (DiffQuick; American Scientific Products, McGraw Park, IL). A total of 200–400 cells was counted from randomly chosen fields using light microscopy (\times 1000). The total number of cells of a particular leukocyte subset was determined by multiplying the percentage of each population in a particular sample by the total number of lung leukocytes collected from each mouse.

Lung macrophage leukotriene analysis

Lung macrophages were purified from total lung leukocytes by adherence to cell culture wells in RPMI 1640 for 1 h. Nonadherent cells were removed by washing with PBS. Ninety percent of the adherent cells were identified as macrophages following staining with DiffQuick. Following overnight culture in RPMI with 10% FCS at 37°C with 5% CO₂ in air, the cell culture medium was recovered and assayed for LTB₄ and cysteinyl-leukotrienes using commercially available EIA kits (Cayman Chemical, Ann Arbor, MI).

Bronchoalveolar lavage

WT and leptin-deficient mice were euthanized by CO₂ inhalation and cannulated through an incision made in the trachea. The lungs were lavaged with 0.5 ml ice-cold HEPES-buffered saline containing EDTA. Following centrifugation at 200 \times g for 5 min, the BALF was stored at -70°C.

Cell culture and isolation

Murine alveolar macrophages were obtained by lung lavage from leptin-deficient and WT mice, as previously described (23). Ninety-five percent of the cells obtained from lavage were identified as macrophages following

staining with DiffQuick. Following lavage fluid centrifugation at 4°C at 200 \times g for 5 min, the cell pellet was resuspended in HBSS and the cells were enumerated using a hemocytometer. The cells were centrifuged a second time and resuspended in RPMI 1640 (Life Technologies, Grand Island, NY). For phagocytosis experiments, 1 \times 10⁵ alveolar macrophages/well were adhered to glass eight-well Falcon culture slides (BD Biosciences, Franklin Lakes, NJ) for 1 h in RPMI 1640.

Serum preparation and opsonization

Specific immune serum was prepared as described previously (26). Before each experiment, 20 \times 10⁷ *K. pneumoniae* were suspended in HBSS in a 5-ml snap-cap tube and opsonized by mixing the bacterial suspension with serum for 15 min at 37°C on a rotating platform. All experiments were performed on targets opsonized with sera at a concentration of 1%.

Phagocytosis of *K. pneumoniae*

Following the addition of 1 \times 10⁶ *K. pneumoniae* opsonized with 1% complete immune serum, the alveolar macrophage cultures were mixed for 1 min with a plate shaker (Hofer Instruments, San Francisco, CA). The alveolar macrophage cultures were then incubated for 30 min at 37°C. Following the incubation period, the extracellular bacteria were removed by washing three times with HBSS. The monolayers containing bacteria were stained with DiffQuick. For each slide, a standard pattern of high powered fields was examined by light microscopy (\times 1000) to enumerate 100 cells. By comparing the phagocytic index in the presence and absence of cytochalasin D (27), we have previously determined that 90% of the cell-associated bacteria using this method were actually internalized (26).

Statistical analysis

Survival curves were evaluated for differences using a log-rank test. Where appropriate, mean values were compared using a paired *t* test or a Kruskal-Wallis test on ranks from nonparametric data. The Dunnett's test or the Student-Newman-Keuls test was used for mean separation. In all cases, a *p* value of <0.05 was considered significant.

Results

Blood, BALF, and lung homogenate leptin levels increase during the course of *K. pneumoniae*

Because previous reports have shown that serum leptin increases in response to inflammatory stimuli, we assessed leptin levels in the lungs, blood, and BALF of WT mice after intratracheal *Klebsiella* challenge (5000 CFU). At baseline, leptin was detectable in all samples, with the lung homogenates having the highest leptin content (3.3 ng/ml) (Fig. 1). Lung homogenate leptin levels increased nearly 3-fold on day 1 and remained elevated 3 days post-*Klebsiella* administration. BALF leptin levels peaked 2 days after *Klebsiella* administration and declined to baseline levels on day 3. Blood leptin levels increased 3-fold 1 day after *Klebsiella* administration and continued to increase 2 and 3 days later.

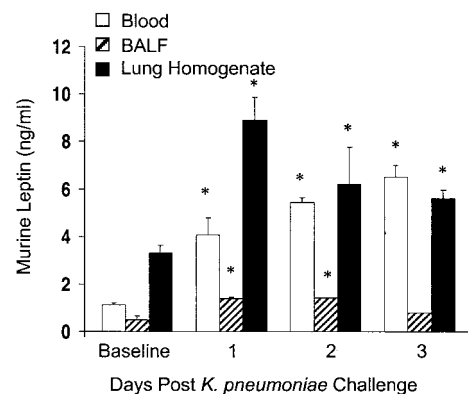


FIGURE 1. Murine leptin levels (in nanograms per milliliter) in blood, BALF, and lung homogenates at baseline and following *K. pneumoniae* challenge in WT mice. Data are presented as the mean \pm SE ($n = 3$ mice per group). *, $p < 0.05$ compared with baseline using a paired *t* test.

Increased mortality in leptin-deficient mice following intratracheal *Klebsiella* challenge

Having demonstrated that leptin is present at the site of infection and increases during the course of *K. pneumoniae*, we next compared survival in WT and leptin-deficient mice to determine whether the absence of leptin influenced mortality. We observed a mortality rate of 40–50% in the WT mice 7 days after *Klebsiella* challenge, which was in accordance with a previously published report (28) (Fig. 2). In contrast, all of the leptin-deficient mice died within 4 days in response to the same bacterial challenge ($p < 0.05$). These data suggest that endogenous leptin plays a protective role in this model of *K. pneumoniae*.

Leptin-deficient mice exhibit impaired bacterial clearance and increased dissemination to the peripheral blood following intratracheal *Klebsiella* challenge

The divergence in the survival curves (Fig. 2) that occurred 2 days following the intratracheal *Klebsiella* challenge suggests that bacterial clearance was impaired in the leptin-deficient mice. To explore this possibility, we determined bacterial CFU 2 days after *K. pneumoniae* challenge and found striking differences in the bacterial burdens of the lungs and the blood. As compared with the WT mice, the bacterial counts in leptin-deficient mice were nearly 2 log-fold greater in the lungs and 3 log-fold greater in the blood (Fig. 3). Moreover, the number of leptin-deficient mice that developed bacteremia was also increased as compared with the WT mice (six of eight vs three of nine, respectively) ($p < 0.05$).

Lung leukocyte number is not altered in leptin-deficient mice

To determine whether differences in lung leukocyte populations were associated with the bacterial clearance impairment in the leptin-deficient mice, the total number and differential counts of lung leukocytes were determined 2 days post-*K. pneumoniae* challenge or saline (control). In comparison with WT mice, there was a greater number of total lung leukocytes in the uninfected leptin-deficient mice (control) (Fig. 4A). While the total number of lung leukocytes increased 2 days after *K. pneumoniae* administration, there was no significant difference between the WT and leptin-deficient mice. All subsets of leukocytes (including macrophages, lymphocytes, and neutrophils) were increased following *K. pneumoniae* administration as compared with saline (Fig. 4B), but there were no significant differences in lung leukocyte subsets between the leptin-deficient and WT mice.

Impaired alveolar macrophage phagocytosis in leptin-deficient mice

We compared alveolar macrophage phagocytosis of opsonized *K. pneumoniae* in vitro to determine whether the impaired bacterial

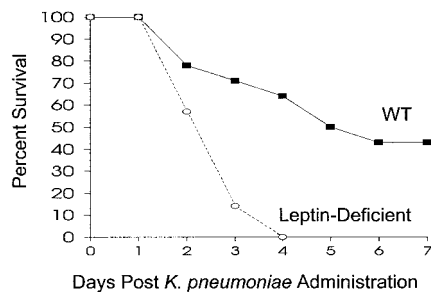


FIGURE 2. Effect of leptin deficiency on survival after *K. pneumoniae* challenge. Leptin-deficient (ovals) or strain-matched WT controls (■) were inoculated via the intratracheal route with 5000 CFU *K. pneumoniae* and monitored over a 7-day period ($n = 14$ per group). Value of $p < 0.05$ in leptin-deficient vs WT mice using the log-rank test.

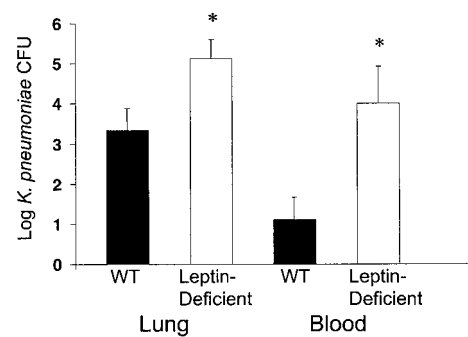


FIGURE 3. Effect of leptin deficiency on lung and blood bacterial burden. Leptin-deficient and WT mice were challenged with 5000 CFU *K. pneumoniae* via the intratracheal route. Two days later, lung homogenate and blood CFU were determined. Bars represent mean \pm SE. $n = 8-9$ mice per group; *, $p < 0.05$ vs WT.

clearance exhibited by the leptin-deficient mice was associated with defective alveolar macrophage phagocytosis. As shown in Fig. 5A, phagocytosis of *K. pneumoniae* in alveolar macrophages from leptin-deficient mice was reduced by ~65% in comparison with the phagocytic index of the alveolar macrophages from WT mice. We next pretreated alveolar macrophages from leptin-deficient mice with exogenous leptin overnight to determine whether the impairment in phagocytosis could be restored. Exogenous leptin enhanced phagocytosis of *K. pneumoniae* in a dose-dependent

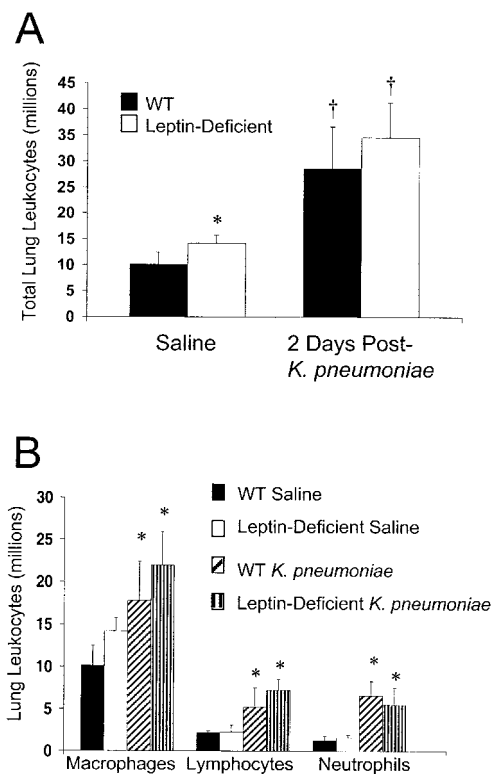


FIGURE 4. A, Total leukocytes obtained from collagenase digestion of the lungs from leptin-deficient and WT mice ($n = 8-9$ mice per group) 2 days following intratracheal saline or *K. pneumoniae* challenge. *, $p < 0.05$ leptin-deficient vs WT after saline; †, $p < 0.05$ saline vs *K. pneumoniae*-treated mice. B, Leukocyte subtype counts obtained from collagenase digestion of the lungs from leptin-deficient and WT mice ($n = 8-9$ mice per group) 2 days following intratracheal saline or *K. pneumoniae* challenge. *, $p < 0.05$ vs saline-treated mice.

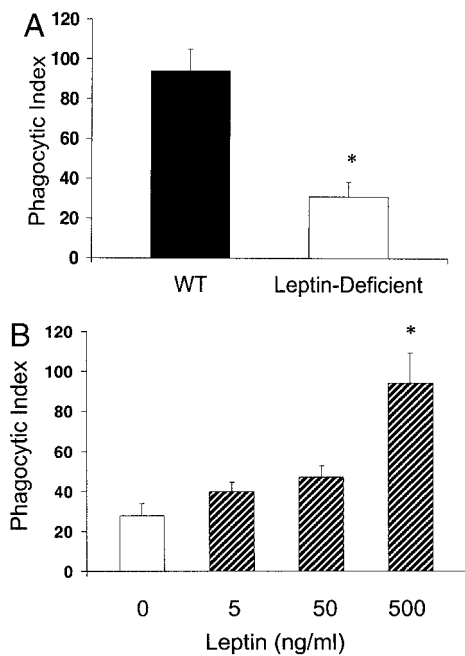


FIGURE 5. A, Alveolar macrophages from leptin-deficient mice exhibit impaired phagocytosis of opsonized *K. pneumoniae* in vitro. Each value represents mean ± SE of three separate experiments. *, $p < 0.05$ vs WT. B, Ability of exogenous leptin to restore phagocytosis of opsonized *K. pneumoniae* in alveolar macrophages from leptin-deficient mice. Alveolar macrophages were pretreated overnight with medium alone or leptin before the addition of opsonized *K. pneumoniae*. Data are expressed as the mean phagocytic index ± SE of three separate experiments. *, $p < 0.05$ vs control (medium alone).

fashion (Fig. 5B), with the 500 ng/ml dose having the greatest enhancement (3-fold increase from baseline).

Leptin deficiency does not impair TNF- α , IL-12, or MIP-2 production in response to K. pneumoniae

Lung homogenate IL-12, TNF- α , and MIP-2 levels were assessed 2 days post-*K. pneumoniae* administrations because these cytokines have been shown to play critical roles in the host defense against *K. pneumoniae*. Although the production of these cytokines increased in response to the bacterial challenge, there were no significant differences between the leptin-deficient and WT mice in any of these cytokines (Table I).

Impaired leukotriene synthesis in macrophages from leptin-deficient mice 2 days after K. pneumoniae administration

Because the leukotrienes have been shown to play a critical role in the host defense against *K. pneumoniae* in mice (23), we assessed leukotriene synthesis in lung macrophages that were harvested 2 days after intratracheal *K. pneumoniae* administration or saline (Fig. 6). While the level of leukotriene B₄ (LTB₄) produced by the

Table I. Lung homogenate cytokine levels

Cytokine (pg/ml)	WT		Leptin-Deficient	
	Saline	<i>K. pneumoniae</i>	Saline	<i>K. pneumoniae</i>
TNF- α	168 ± 67	410 ± 141	174 ± 36	555 ± 337
IL-12	374 ± 128	3089 ± 85 ^a	174 ± 35	2783 ± 235 ^a
MIP-2	ND ^b	1024 ± 58 ^a	ND	980 ± 211 ^a

^a Value of $P < 0.05$.

^b ND, Not detected.

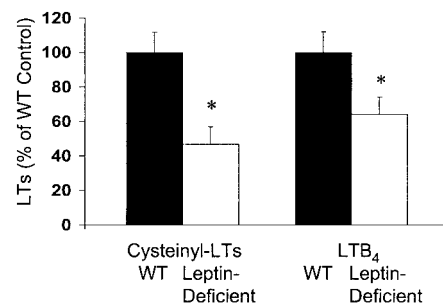


FIGURE 6. Reduced leukotriene (LT) synthesis in lung macrophages 2 days after *K. pneumoniae* administration. Lung macrophages were obtained from leptin-deficient and WT mice 2 days post-*K. pneumoniae* challenge. Values were normalized to that of the WT mice and represent the mean ± SE ($n = 5$). *, $p < 0.05$ vs WT.

macrophages harvested from saline-treated mice was below the detection limit of the EIA kit, the level of cysteinyl-leukotriene synthesis was 79 (WT) and 89 (leptin-deficient) pg/1 × 10⁶ cells. Leukotriene synthesis was increased above the saline-treated levels in the macrophages obtained from WT and leptin-deficient mice 2 days after *K. pneumoniae* administration. Leukotriene synthesis in the macrophages from WT mice was 5177 pg for the cysteinyl-leukotrienes and 50 pg for LTB₄. This is consistent with the fact that the major 5-lipoxygenase product of murine macrophages is cysteinyl-leukotrienes, which are produced at levels that are 10-fold greater than LTB₄. In comparison with macrophages harvested from WT mice, cysteinyl-leukotriene and LTB₄ synthesis were reduced by 50 and 35%, respectively, in macrophages obtained from the leptin-deficient mice.

Exogenous leptin restores cysteinyl-leukotriene synthesis in macrophages from leptin-deficient mice

Because leukotriene synthesis was reduced in macrophages from leptin-deficient mice, we explored the possibility that exogenous leptin could reverse this defect. Overnight pretreatment of peritoneal macrophages with leptin augmented maximally stimulated cysteinyl-leukotriene synthesis in response to a calcium ionophore in a dose-dependent manner (Fig. 7). A very high concentration of leptin (500 ng/ml) was required to completely restore cysteinyl-leukotriene synthesis in these cells. These data demonstrate that

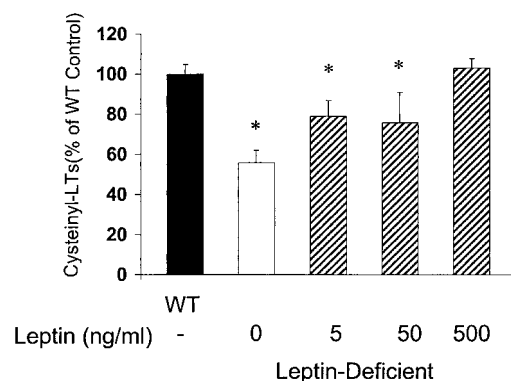


FIGURE 7. Exogenous leptin reconstitutes cysteinyl-leukotriene (cysteinyl-LT) synthesis in peritoneal macrophages. Overnight pretreatment of peritoneal macrophages from WT or leptin-deficient mice with either medium or leptin. Each value represents the mean ± SE of three separate experiments. *, $p < 0.05$ vs WT.

defective cysteinyl-leukotriene synthesis in macrophages from leptin-deficient mice is, at least in part, a consequence of leptin deficiency.

Discussion

In the present study, we investigated the role of leptin in Gram-negative bacterial pneumonia by comparing the responses of WT and leptin-deficient mice after an intratracheal challenge with *K. pneumoniae*. To determine whether leptin might play an important role in host defense against bacterial pneumonia, we first asked whether leptin was present in the lungs during Gram-negative pneumonia. We observed measurable leptin levels in the lungs of control mice and increased levels in lung homogenates and BALF following *K. pneumoniae* administration. This finding is important because it is the first demonstration (to our knowledge) of increased lung leptin levels in response to bacterial pneumonia. While we cannot exclude the possibility that leptin synthesis occurs in the lung, there is no evidence to date that lung cells are capable of doing so. Therefore, we suggest that the most likely explanation for increased leptin in the BALF and lung homogenates following *K. pneumoniae* challenge is that leptin is produced by adipocytes in response to the *K. pneumoniae* infection and was secreted into the peripheral circulation. The fact that leptin increased in serum following *Klebsiella* administration was not surprising because leptin has been reported to increase in the serum of laboratory animals following in vivo administration of LPS, IL-1, TNF- α , or bacteria and with bacterial peritonitis and sepsis (8, 20, 29, 30). However, there is controversy over whether plasma leptin remains elevated during infection. While Bornstein et al. (21) and Francisco et al. (31) reported increased plasma leptin levels in patients with sepsis, Carlson et al. (32) reported similar fasting plasma leptin levels in septic patients and controls. In addition, Grunfeld et al. (33) reported that leptin levels do not increase in HIV infection and during secondary infection in patients with AIDS. In contrast to studies that have described an acute increase in serum leptin following inflammatory stimuli (29, 34), we observed a sustained increase in blood and lung leptin levels following *K. pneumoniae* challenge. It would be anticipated that leptin, which is a relatively small protein (16 kDa), might leak into the lung interstitium and alveolar space along with other plasma proteins. Interestingly, the accumulation of leptin at a site of inflammation has also been seen in human patients with endometriosis (35).

Having established the presence of leptin in the lungs during the course of bacterial pneumonia, we next conducted survival studies to determine whether the absence of leptin would influence survival following the administration of a dose of bacteria that results in 50% mortality in WT mice. The early mortality (within 4 days of bacterial challenge) and impaired bacterial clearance with increased bacteremia observed in the leptin-deficient mice suggest that the presence of leptin is required for an effective innate immune response. This finding is noteworthy because it is the first (to our knowledge) to indicate that leptin-deficient mice exhibit increased susceptibility to Gram-negative bacterial pneumonia. While the total lung leukocyte counts were greater in leptin-deficient as compared with the WT mice treated with saline, we did not observe any significant differences in the types or numbers of cells recruited to the lungs at a critical time point after *K. pneumoniae* challenge. Interestingly, Faggioni et al. (36) found elevated peripheral blood monocyte and neutrophil counts in leptin-deficient as compared with WT mice. Based on this evidence, it does not appear that the defect in host defense observed in leptin-deficient mice was related to an impairment in the recruitment of inflammatory cells.

The most credible explanation for the reduced bacterial clearance and increased mortality observed in the leptin-deficient mice in response to pulmonary *K. pneumoniae* infection is defective alveolar macrophage function. The fact that pretreatment of alveolar macrophages with exogenous leptin, at a very high concentration (500 ng/ml), reversed the phagocytic defect indicates that this impairment was not due to developmental abnormalities. An effective host response against *K. pneumoniae* requires rapid clearance of this organism from the lower respiratory tract. The alveolar macrophage plays a critical role in the early innate immune response by phagocytosing and killing bacteria that it encounters in the alveolar milieu. In previous reports, depletion of alveolar macrophages or defective alveolar macrophage phagocytosis was associated with reduced bacterial clearance and enhanced lethality in *K. pneumoniae*-infected mice (23, 37). Finally, the fact that the alveolar macrophage phagocytosis was impaired in leptin-deficient mice confirms previous reports that have shown impaired phagocytosis in peritoneal macrophages and bone marrow cells from leptin-deficient mice (17, 18).

Following ingestion of bacteria, macrophages elaborate inflammatory mediators that enhance the phagocytic capabilities of nearby macrophages. Previously, we have shown that alveolar macrophages synthesize leukotrienes during phagocytosis of *K. pneumoniae* and that phagocytosis is impaired when leukotriene synthesis is inhibited in alveolar macrophages by either pharmacologic or genetic means (26). Moreover, leukotriene-deficient mice exhibit enhanced lethality that is associated with impaired alveolar macrophage phagocytosis (23). While we have not exhausted the possibility that other functional abnormalities may exist in macrophages from leptin-deficient mice, it is likely that impaired leukotriene synthesis contributes to the observed defect in phagocytosis. The link between leptin and leukotriene synthesis is also novel, and the mechanism responsible for this observation remains to be explored. Interestingly, others have demonstrated impaired leukotriene synthesis in malnutrition (38, 39). We did not find differences between the WT and leptin-deficient mice in lung homogenate TNF- α , IL-12, and MIP-2. This is noteworthy because these cytokines have been shown to be protective in the murine model of *K. pneumoniae* (40–42).

It is possible that other phenotypic abnormalities recognized in the leptin-deficient mice may have contributed to the observed host defense impairment against *K. pneumoniae*. Obesity may have been an important factor that may have contributed to the observed host defense impairments in the leptin-deficient mice. However, this seems unlikely because obesity was not a significant risk factor for nosocomial pneumonia in human patients (43–45). Of the abnormalities associated with the leptin-deficient mice, excess glucocorticoid synthesis might have the most potent immune suppressive effects. While others have shown that glucocorticoids can impair neutrophil recruitment to sites of inflammation and inhibit TNF- α and IL-12 synthesis (46, 47), we did not observe any differences between the WT and leptin-deficient mice in these end points. Furthermore, no alterations in host defense against bacterial peritonitis were observed in transgenic mice overexpressing corticotropin-releasing hormone with increased circulating levels of corticosterone (48). One might assume that elevated glucocorticoids associated with the leptin-deficient phenotype may theoretically explain impaired leukotriene synthesis in macrophages. However, Sebaldt et al. (49) previously reported that a high dose of glucocorticoids (60 mg prednisone/day) was required to inhibit leukotriene synthesis in macrophage-rich BALF recovered from human subjects. In addition, Riddick et al. (50) have shown that pretreatment of monocytes with dexamethasone, at physiologic

levels, actually increased calcium-ionophore-stimulated leukotriene synthesis. Finally, we were able to demonstrate that exogenous leptin, at a very high dose, completely restored macrophage leukotriene synthesis in vitro. This suggests that leptin itself may enhance leukotriene synthesis independent of the potential effect of elevated glucocorticoids in the leptin-deficient mouse. It is also important to note that defective alveolar macrophage phagocytosis of bacteria observed in the leptin-deficient mice cannot be explained by excess glucocorticoids, because dexamethasone pretreatment has been shown to enhance macrophage phagocytosis and killing of bacteria in vitro (51). Therefore, the most likely explanation for the host defense impairments against *K. pneumoniae* in leptin-deficient mice was due to the absence of leptin itself.

While the restoration of phagocytosis in alveolar macrophages from leptin-deficient mice in vitro required a supraphysiologic dose (500 ng/ml) of leptin in vitro, it is likely that leptin may confer other protective effects in vivo. Leptin has been shown to polarize Th cells toward a Th1 phenotype (19), prevent lymphoid atrophy and reconstitute lymphoid cellularity during starvation in mice (7), and restore circulating lymphocyte populations in *ob/ob* mice (36). It seems likely that leptin can augment the synthesis of T cell factors, such as IFN- γ , during the course of bacterial pneumonia that could enhance macrophage effector functions. This is noteworthy because impaired cell-mediated immunity is a prominent characteristic of immune suppression in malnourished animals.

In summary, we observed that increases in systemic and lung leptin levels accompany infection. This increase in leptin is critical for host defense because leptin-deficient mice exhibited increased susceptibility to a pulmonary Gram-negative bacterial challenge. This enhanced lethality in the leptin-deficient mice was associated with impaired bacterial clearance and reduced alveolar macrophage phagocytosis of *K. pneumoniae* in vitro. Leukotrienes are important for optimal macrophage phagocytosis, and we observed reduced leukotriene synthesis in lung macrophages from leptin-deficient mice. Using peritoneal macrophages, the defect in cysteinyl-leukotriene synthesis in the leptin-deficient mice could be reversed by overnight pretreatment with exogenous murine leptin at a very high concentration. These results indicate that leptin is an essential component of pulmonary antibacterial host defense in mice, and suggest that leukotrienes are important mediators of the leptin effect. While an important role for leptin in human pulmonary host defense has not been established, it is noteworthy that individuals who are most susceptible to bacterial pneumonia (the elderly, the HIV infected, the malnourished, and diabetics) exhibit altered leptin secretion or responsiveness as well as reduced leukotriene synthesis (38, 39, 52, 53).

Acknowledgments

We thank Thomas Moore for his technical advice.

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