Leptin deficiency enhances sensitivity to endotoxin-induced lethality

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Faggioni, Raffaella, Giamila Fantuzzi, Cem Gabay, Arthur Moser, Charles A. Dinarello, Kenneth R. Feingold, and Carl Grunfeld. Leptin deficiency enhances sensitivity to endotoxin-induced lethality. Am. J. Physiol. 276 (Regulatory Integrative Comp. Physiol. 45): R136–R142, 1999.—Leptin is induced by lipopolysaccharide (LPS) and cytokines. We investigated the role of leptin in LPS-induced toxicity using leptin-deficient (ob/ob) and leptin receptor-deficient (db/db) mice. Sensitivity to LPS-induced mortality is significantly greater in ob/ob mice compared with their own lean littermates but not in db/db mice. LPS reduced serum glucose in both ob/ob and db/db mice but induced corticosterone only in db/db mice. Despite the very high basal levels of serum leptin in db/db mice, a twofold increase in serum leptin levels was observed after LPS in both db/db mice and their lean littermates. No differences were detected in LPS-induced serum levels of interleukin (IL)-1β, tumor necrosis factor, macrophage inflammatory protein-1α, and interferon-γ in ob/ob mice compared with their own littermates. In contrast, a blunted induction of IL-10 and IL-1 receptor antagonist (IL-1Ra) was observed in ob/ob mice compared with their littermates. In vitro, leptin induced IL-1Ra production and upregulated the IL-1Ra induction by LPS in macrophages. Moreover, treatment with leptin reversed the increased sensitivity to LPS-induced lethality found in ob/ob mice. These results suggest that leptin participates in the host response to inflammation by modulating the host immune and cytokine responses after LPS.

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MATERIALS AND METHODS

Animals and treatments. Female C57BL/6J ob/ob, C57BL/KsJ db/db (both weighing ~40 g), and their respective lean littermates (+/+) were purchased from Jackson Laboratory (Bar Harbor, ME). Lean littermate control mice were not separated by genotype (either +/+ and +/ob or +/+ and +/db) and are therefore referred to as +/ob for the ob/ob littermates and +/db for the db/db littermates. For analysis of serum cytokine, glucose, and corticosterone levels, LPS (Escherichia coli 055:B5; Difco Laboratories, Detroit, MI) was administered intraperitoneally at 5 µg/g. Control mice received intraperitoneal saline. Blood was collected from the retroorbital plexus under halothane anesthesia at 0, 2, and 6 h after LPS administration. For lethality studies, LPS was injected intraperitoneally at doses of 10, 30, 100, 300, and 1,000 µg/mouse, as indicated in the text. The ob/ob mice were given five injections (~40, ~16, -1, +8, and +32 h) of 100 µg of murine leptin (Amgen, Thousand Oaks, CA) or saline surrounding challenge with 150 µg (5 µg/g) of LPS (time 0). These studies were approved by the Animal Studies Committee of the San Francisco Veterans Affairs Medical Center.

Cell culture. The RAW 264.7 murine macrophage cells, obtained from American Type Tissue Collection (Rockville, MD), were seeded (10⁴/well) in 24-well plates in RPMI 1640 (Mediatech, Herndon, VA) supplemented with penicillin, streptomycin, and 10% of FCS (Summit Biotechnology, Greeley, CO). After 4 h of culture, the medium was replaced by fresh RPMI 1640 media supplemented with 2.5% FCS, and stimulants were added. The culture supernatants were collected after 24 h and kept frozen until assayed for their IL-1 receptor antagonist (IL-1Ra) content. The leptin used in the RAW 264.7 experiments was from R&D Systems (Minneapolis, MN).

Cytokine measurement. IL-1β, interferon-γ (IFN-γ), IL-10, and IL-6 levels were measured with ELISA kits specific for murine cytokines, obtained from Endogen (Cambridge, MA). Tumor necrosis factor-α (TNF-α) was measured using an electrochemiluminescence method using the ORIGEN apparatus (Ingen, Gaithersburg, MD), as previously described (12). Macrophage inflammatory protein-1α (MIP-1α) was measured with the same technique using anti-MIP-1α antibodies and standards from R&D Systems. A previously described sandwich ELISA was used to determine IL-1Ra concentration (14).

Other assays. Serum leptin levels were measured using a radioimmunoassay kit specific for mouse leptin (Linco Research, St. Charles, MO). Serum glucose was measured using a kit from Sigma Chemical. Serum corticosterone was measured using a kit from ICN Pharmaceuticals (Costa Mesa, CA).

Statistical analysis. Analysis of variance with Bonferroni post hoc test was used. Data are expressed as means ± SE. Statistical significance for lethality rates was determined by Fisher’s exact test.

RESULTS

LPS-induced lethality in ob/ob and db/db mice and leptin induction in db/db mice. The ob/ob and db/db mice and their own lean littermates (+/+) were injected with increasing doses of LPS. As shown in Fig. 1A, the sensitivity to the lethal effect of LPS was significantly enhanced in ob/ob mice compared with +/ob mice, with an LPS half-maximal lethal dose (LD₅₀) of 50 µg for ob/ob and 600 µg for +/ob mice. When the difference in the body weight between ob/ob and +/ob mice is taken into account, the LD₅₀ values for LPS are 1.25 mg/kg for the ob/ob and 30 mg/kg for the +/ob mice, a 24-fold increase in sensitivity. Mice died between 24 and 48 h after treatment for both groups.

As shown in Fig. 1B, doses of LPS between 100 and 1,000 µg caused similar dose-dependent lethality in db/db and +/db mice, with an LPS LD₅₀ of 200 µg for both db/db and +/db mice. When adjusted per body weight, the LD₅₀ was 5 mg/kg for db/db and 10 mg/kg for the +/db mice, showing only a two-fold increased...
sensitivity between db/db and +/?db mice compared with the 24-fold difference observed between ob/ob and +/?ob mice.

Administration of LPS increases leptin levels in lean mice. The ob/ob mice do not have circulating leptin, whereas db/db mice have very high levels of leptin. As shown in Fig. 2, despite the very high levels of serum leptin in db/db mice, a twofold induction of leptin levels after LPS was observed in both db/db and +/?db mice.

Glucose and corticosterone levels in ob/ob and db/db mice after LPS administration. As expected, ob/ob and db/db mice display basal hyperglycemia (Fig. 3) and high serum corticosterone levels (Fig. 4) compared with their littermates, with the ob/ob having lower basal glucose levels and higher corticosterone levels than those of the db/db mice. After LPS administration (5 µg/g), significant reductions in serum glucose levels were observed in ob/ob and db/db mice and their lean littermates. These data indicate that both ob/ob and db/db mice are sensitive to the hypoglycemic effects of LPS.

Basal corticosterone levels were higher in both ob/ob and db/db mice compared with lean littermates. As shown in Fig. 4A, the already high basal corticosterone levels in ob/ob mice were not increased further by LPS administration. In contrast, in db/db mice, serum corticosterone levels were increased by LPS and reached similar levels as those induced in +/?db mice (Fig. 4B).

The failure of LPS to increase serum corticosterone levels in ob/ob mice raised the possibility that an absence of corticosterone feedback inhibition of proinflammatory cytokine production in ob/ob mice could account for the increased sensitivity to LPS. Therefore, we next determined whether circulating cytokine levels were higher after LPS administration in ob/ob mice compared with +/?ob mice.

Circulating cytokine levels in ob/ob mice after LPS administration. The ob/ob mice and their littermates were administered 5 µg/g of LPS, and blood was collected 8 h for cytokine measurements. *P < 0.05, LPS vs. saline by t-test.

Fig. 2. Serum leptin levels in db/db mice after LPS administration. The db/db and +/?db mice were administered 5 µg/g of LPS or saline, and blood was collected at 8 h for leptin measurements. *P < 0.05, LPS vs. saline by t-test.

Fig. 3. Serum glucose (Glu) levels after LPS administration. A: +/?ob and ob/ob mice were injected ip with LPS (5 µg/g). Blood was collected at 0, 2, and 6 h after LPS administration, and serum glucose levels were measured. Data are means ± SE (n = 5). ANOVA has been used to compare ob/ob with +/?ob mice at each time point. **P < 0.01 and ***P < 0.001, ob/ob vs. +/?ob. B: +/?db and db/db mice were injected ip with LPS (5 µg/g). Blood was collected at 0, 2, and 6 h after LPS administration, and serum glucose levels were measured. Data are means ± SE (n = 5). ANOVA has been used to compare db/db with +/?db mice at each time point. ***P < 0.001, db/db vs. +/?db.

Fig. 4. Serum cytokine levels after LPS administration. A: Serum IL-1β, NF-kB, IFN-γ, and MIP-1α were measured in ob/ob and +/?ob mice. Serum IL-6 levels measured 2 h after LPS were significantly lower in ob/ob than in +/?ob mice (P < 0.05 by t-test). However, 6 h after LPS, the levels of serum IL-6...
were increased to a similar degree in both groups of mice.

We next measured the levels in the serum of the anti-inflammatory IL-10 and IL-1RA. Significantly lower levels of IL-10 (Fig. 5A) and IL-1RA (Fig. 5B) were induced by LPS in ob/ob compared with +/+ob mice.

In vitro effect of leptin on IL-1Ra production in macrophages. RAW 264.7 murine macrophage cells were stimulated in vitro for 24 h with 10 ng/ml of LPS, 1 µg/ml of leptin, or the combination, and then IL-1Ra secretion was measured. The leptin dose is comparable to that used to show in vitro effects of leptin on hematopoietic cells, on proinflammatory cytokine production, and on mitogen-activated protein kinase (21, 27, 29). As shown in Fig. 6, leptin induced an increase in IL-1Ra secretion from macrophages and potentiated the production of IL-1Ra after LPS stimulation, indicating that leptin can directly modulate the IL-1Ra production by macrophages.

Effect of leptin treatment on LPS-induced lethality in ob/ob mice. Because there is no circulating leptin in ob/ob mice, we next assessed the possibility that the absence of leptin enhanced sensitivity to LPS-induced lethality. The ob/ob mice were treated with five injections (−40, −16, −1, +8, and +32 h) of 100 µg of leptin or saline surrounding challenge with 150 µg (5 µg/g) of LPS (administered at time 0). The 2-day pretreatment with leptin caused a 40% decrease in food intake but did not induce a significant loss of body mass in that time period (initial body weight 38.6 ± 0.5; body weight after 2 days pretreatment 37.0 ± 0.5). As shown in Fig. 7, survival was prolonged in leptin-treated ob/ob mice compared with saline-treated controls. At 2 and 3 days after LPS administration, leptin treatment resulted in a statistically significant protection against LPS-induced lethality compared with the saline treatment.

DISCUSSION

In this report, we have studied the role of leptin in the inflammatory and toxic responses elicited by LPS administration using leptin-deficient (ob/ob) and leptin receptor-deficient (db/db) mice. Sensitivity to the lethal effect of LPS was markedly enhanced in ob/ob compared with +/+ob mice, in agreement with recent data showing increased sensitivity to LPS-induced anorexia and liver injury in ob/ob mice compared with +/+ob mice (11, 32). Short-term leptin treatment reverses the increased sensitivity to LPS observed in ob/ob mice before significant weight loss occurred, indicating that the increased sensitivity to LPS-induced lethality was due to the leptin deficiency.

Table 1. Serum cytokine levels after LPS administration in ob/ob and +/+ob mice

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>2 h LPS</th>
<th>6 h LPS</th>
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</thead>
<tbody>
<tr>
<td>IL-1β, pg/ml</td>
<td>57.8 ± 15.0</td>
<td>62.0 ± 7.4</td>
</tr>
<tr>
<td>TNF, pg/ml</td>
<td>6.5 ± 0.3</td>
<td>1.3 ± 0.2</td>
</tr>
<tr>
<td>IFN-γ, ng/ml</td>
<td>&lt;0.07</td>
<td>13.0 ± 3.1</td>
</tr>
<tr>
<td>MIP-1α, ng/ml</td>
<td>1.76 ± 0.09</td>
<td>0.56 ± 0.09</td>
</tr>
<tr>
<td>IL-6, ng/ml</td>
<td>68.4 ± 6.1</td>
<td>51.8 ± 13.2</td>
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</tbody>
</table>

Data are means ± SE, n = 5 mice for each group. LPS, lipopolysaccharide; IL, interleukin; TNF, tumor necrosis factor; IFN-γ, interferon-γ; MIP-1α, macrophage inflammatory protein-1α. No cytokines were detected in the serum of saline-treated mice. *P < 0.05.
In contrast to ob/ob mice, db/db mice and their lean littermates had a similar sensitivity to LPS-induced lethality. These data eliminate the possibility that the increased sensitivity to LPS-induced toxicity observed in ob/ob mice was due to obesity, because both ob/ob and db/db mice are equally obese. Moreover, db/db mice are even more diabetic than ob/ob mice (9); therefore, diabetes cannot account for these differences. Furthermore, the profound decrease in serum glucose levels after LPS administration detected in both ob/ob and db/db mice indicates that the difference observed in terms of LPS-induced lethality was not due to a more generalized defect in the responses of ob/ob mice to LPS.

We further showed that leptin administration reverses the increased sensitivity to LPS observed in ob/ob mice. These results, together with the previous observations that inflammatory stimuli, like LPS, turpentine, or proinflammatory cytokines, increase leptin production (10, 17, 19, 26) suggest that leptin is involved in the acute phase response to tissue injury and

Fig. 5. Serum interleukin (IL)-10 and IL-1 receptor antagonist (Ra) levels after LPS administration. A: +/+ob and ob/ob mice were injected ip with LPS (5 µg/g). Blood was collected at 0, 2, and 6 h after LPS administration, and serum IL-10 levels were measured. Data are means ± SE (n = 5). ANOVA has been used to compare ob/ob with +/+ob mice at each time point. ***P < 0.001, ob/ob vs. +/+ob. B: +/+ob and ob/ob mice were injected ip with LPS (5 µg/g). Blood was collected at 0, 2, and 6 h after LPS administration, and serum IL-1Ra levels were measured. Data are means ± SE (n = 5). ANOVA has been used to compare ob/ob with +/+ob mice at each time point. *P < 0.05 and **P < 0.01, ob/ob vs. +/+ob.

Fig. 6. In vitro effect of leptin on IL-1Ra production in macrophages. RAW murine macrophage cells were stimulated with 10 ng/ml of LPS, 1 µg/ml of leptin, or the combination. Supernatant was collected 24 h after treatment, and IL-1Ra levels were determined. Results are means ± SE (n = 4) of 2 experiments performed in duplicate and are expressed as % of IL-1Ra levels measured in controls. IL-1Ra levels (ng/ml) in the control group were 1.57 ± 0.13 in one experiment and 3.07 ± 0.15 in the other. *P < 0.05 and ***P < 0.01 vs. control. O○P < 0.01 vs. LPS by ANOVA.

Fig. 7. Effect of leptin treatment on LPS-induced lethality in ob/ob mice. The ob/ob mice were given 5 injections (-40, -16, -8, +32 h) of 100 µg of leptin or saline surrounding challenge with 150 µg (5 µg/g) of LPS (time 0). Mortality was assessed daily for 7 days. *P < 0.05 and **P < 0.01 vs. saline/LPS by Fisher’s exact test.
systemic inflammation. It is noteworthy that leptin has structural similarities to the IL-6-like cytokine family, which also includes IL-11, CNTF, and LIF (33). Additionally, the leptin receptor is a member of the gp-130 family (28, 33). In vivo, protection against lethal endotoxic-septic shock can be induced by prior administration of CNTF or LIF (2, 31). However, all of these cytokines provide protection against LPS-induced lethality by decreasing the induction of TNF. In this study, we have shown that LPS induced comparable levels of IL-1α, TNF-α, IFN-γ, MIP-1α, and IL-6 in ob/ob and +/+ob mice. Interestingly, lower levels of IL-10 and IL-1Ra were detected after LPS in ob/ob mice compared with +/+ob mice. In addition, we have shown that leptin stimulates IL-1Ra secretion by macrophages and potentiates the LPS-induced production of IL-1Ra, indicating that leptin has direct effects on the immune system. The blunted production of IL-10 and IL-1Ra by LPS in ob/ob mice could contribute to the increased toxicity of LPS but is unlikely to fully account for that increased sensitivity, and other cytokines are probably also involved.

Interestingly, it has been reported recently that, in critically ill, septic patients, leptin levels were increased significantly and were higher in survivors than in nonsurvivors, suggesting that leptin might have a protective effect in patients with severe inflammatory disease (4).

The activation of the HPA axis is a component of the host response to inflammation and provides an important negative feedback to proinflammatory cytokine production and toxicity (3). A failure of LPS to increase the already high serum corticosterone levels was observed in ob/ob mice. It is therefore possible that the inability of ob/ob mice to adequately respond to LPS in terms of HPA axis activation might also contribute to the increased susceptibility to LPS-induced toxicity. A direct effect of leptin on development and differentiation of hemopoietic lineages has been described (8). Leptin has also been shown to enhance phagocytosis by macrophages (15). Furthermore, we now present direct evidence that leptin modulates IL-1Ra secretion by macrophages both in basal and LPS-stimulated states. Our results show that a defect in leptin production is associated with an impaired host response to inflammation. The blunted production of IL-1Ra and IL-10 and the dysregulation in corticosterone induction in leptin-deficient mice are examples of immunological changes that could contribute to the increased susceptibility to LPS-induced lethality.

The ob/ob mice do not have leptin, whereas very high serum levels of leptin are detectable in db/db mice (5, 18, 24) and are further increased after LPS. The db/db mice are resistant to the weight-regulating effect of leptin because they have a defect in the Ob-Rb form of the leptin receptor (7, 20). However, the other forms of the leptin receptor, which are highly expressed in peripheral tissues, are normally expressed in db/db mice. The Ob-Ra form has recently been shown to be capable of transmitting signals and might mediate some of the extrahypothalamic activities of leptin (23). Our results raise the possibility that circulating leptin acting through the Ob-Ra contributes to the host response to inflammation, accounting for the difference in LPS sensitivity observed when ob/ob and db/db mice are compared with their respective lean littermates.

Leptin induction during inflammation appears to be part of the cytokine cascade, which is activated during the acute phase response to infection and injury. We have previously shown that leptin levels are increased by cytokines, such as IL-1, TNF, and IL-6. We now report that leptin deficiency is accompanied by increased susceptibility to LPS-induced lethality and decreased induction of anti-inflammatory cytokines, supporting the hypothesis that leptin is an important cytokine component of the acute phase response. Recognizing the ability of leptin to modulate cytokine production can also help in understanding the biological basis for the pleiotropy of the activities of leptin (25). Given that leptin functions as a cytokine, we would speculate that disorders characterized by decreased leptin levels, such as cachexia and starvation, might result in an impaired host defense that would increase susceptibility to infection.

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REFERENCES


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Faggioni, R., G. Fantuzzi, J. Fuller, C. A. Dinarello, K. R.
11.
Faggioni, R., J. Fuller, A. Moser, K. R. Feingold, and C.
14.
15.
Ghilardi, N., S. Ziegler, A. Wiestner, R. Stoffel, M. H. Heim,
17.
Grunfeld, C., C. Zhao, J. Fuller, A. Pollack, A. Moser, J.
19.
Janik, J. E., B. D. Curti, R. V. Considine, H. C. Rager, G. C.
Lee, G. H., R. Proenca, J. M. Montez, K. M. Carroll, J. G.
18.
Lee, G. H., R. Proenca, J. M. Montez, K. M. Carroll, J. G.
19.
Lee, G. H., R. Proenca, J. M. Montez, K. M. Carroll, J. G.
20.
Lee, G. H., R. Proenca, J. M. Montez, K. M. Carroll, J. G.
21.
Loffreda, S., S. Q. Yang, H. Z. Lin, C. L. Karp, M. L.
Brenghman, D. J., Wang, A. S. Klein, G. B. Bulkley, C. Bao,
22.
Montague, C. T., I. S. Farooqi, J. P. Whitehead, M. A. Soos,
H. Rau, N. J. Wareham, C. P. Sewter, J. E. Digby, S. N.
23.
Murakami, T., T. Yamashita, M. Iida, M. Kuwajima, and K.
24.
Pelleymounter, M. A., M. J. Cullen, M. B. Baker, R. Hecht, D.
25.
26.
J askowici, D. Rivet, J. S. Flier, B. B. Lowell, D. L. Fraker,
27.
Takahashi, Y., Y. Okimura, I. Mizuno, K. Iida, T. Takahashi,
28.
Tartaglia, L. A., M. Dembski, X. Weng, N. Deng, J. Culppeper,
29.
Umemoto, Y., K. Tsuji, F. C. Yang, Y. Ebihara, A. Kaneko, S.
30.
Vaisse, C., J. L. Halaas, C. M. Horvath, J. J. Darnell, M.
31.
32.
Yang, S. Q., H. Z. Lin, M. D. Lane, M. Clemens, and A. M.
33.
Zhang, F., M. B. Basinski, J. M. Beals, S. L. Briggs, L. M.
Churgay, D. K. Clawson, R. D. DiMarchi, T. C. Furman,
J. E. Hale, H. M. Hsiung, B. E. Schoner, D. P. Smith, X. Y.