Role of Leptin Deficiency in Early Acute Renal Failure during Endotoxemia in ob/ob Mice

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Abstract. It is known that, among human patients with sepsis, acute renal failure (ARF) dramatically increases mortality rates to 50 to 80%. However, the pathogenesis of septic ARF is not fully understood. An increase in endotoxin-induced mortality rates for leptin-deficient ob/ob mice was recently demonstrated. In comparison with ob/ob mice, db/db mice, which are deficient in the long isoforms of leptin receptors (Ob/Rb), demonstrate lower mortality rates after exposure to the endotoxin LPS. In db/db mice, mRNA for the short isoforms of leptin receptors is constitutively expressed in the kidney, lung, liver, and macrophages. It is known that plasma leptin levels increase in rodents after exposure to LPS, and this finding has been demonstrated for db/db mice. Because ob/ob and db/db mice are both obese, factors other than obesity must be involved in the increased mortality rates for ob/ob mice. In this study, the hypothesis that the short forms of leptin receptors might offer protection against endotoxin-induced lethality at least in part by providing protection against ARF was examined. Serum leptin levels were significantly increased with LPS treatment in wild-type and db/db mice but not ob/ob mice. GFR decreased significantly 16 h after the homozygous ob/ob mice received intraperitoneal injections of 0.3 mg/kg LPS (0.37 ± 0.04 ml/min per g kidney versus 0.83 ± 0.06 ml/min per g kidney, n = 6, P < 0.01); the mean arterial pressure (MAP) remained unchanged. For ob/ob littermates (+/+ob), there was no significant change in either MAP or GFR when the mice were challenged with the same time interval (16 h) and dose of LPS. In contrast to ob/ob mice, there was no significant change in GFR or MAP when homozygous db/db mice or their littermates received injections of an even higher dose of LPS (0.4 mg/kg). Mouse recombinant leptin had no effect on GFR when ob/ob mice received 0.3 mg/kg LPS injections. However, renal function (serum creatinine levels, 0.4 ± 0.1 mg/dl versus 0.9 ± 0.1 mg/dl, P < 0.01) and MAP (68 ± 4 mmHg versus 51 ± 2 mmHg, n = 6, P < 0.01) were significantly improved with leptin replacement when the ob/ob mice developed hypotensive ARF with a higher dose of LPS (0.5 mg/kg). In summary, the previously reported increased susceptibility to LPS of ob/ob mice, compared with db/db mice, may be attributable at least in part to increased susceptibility to ARF.

Severe sepsis and septic shock are common and are associated with significant mortality rates and substantial consumption of health care resources. There are an estimated 751,000 cases of sepsis or septic shock in the United States each year, and those conditions are responsible for as many deaths each year as are acute myocardial infarctions (215,000 deaths) (1). It is known that, among human patients with sepsis, acute renal failure (ARF) dramatically increases mortality rates to 50 to 80% (2). However, the pathogenesis of septic ARF is not fully understood. An increase in endotoxin-induced mortality rates for leptin-deficient ob/ob mice was recently demonstrated. In comparison with ob/ob mice, db/db mice, which are deficient in the long isoforms of the leptin receptor (Ob/Rb), demonstrated lower mortality rates with exposure to the endotoxin LPS (3). In db/db mice, mRNA for other short isoforms of leptin receptors is constitutively present in the kidney, lung, liver, and macrophages (4). It is known that plasma leptin levels increase in rodents after exposure to LPS, and this finding has been demonstrated in db/db mice (5,6). Because ob/ob and db/db mice are both obese, factors other than obesity must be involved in the increased mortality rates for ob/ob mice. In this study, the hypothesis that the short isoforms of leptin receptors might offer protection against endotoxin-induced lethality at least partly by providing protection against ARF was examined. To test our hypothesis, this study was undertaken to examine renal function during endotoxemia in ob/ob and db/db mice.

Materials and Methods

Animals
The experimental protocol was approved by the Animal Ethics Review Committee of the University of Colorado Health Sciences Center. Wild-type (C57BL/6), ob/ob (B6.V-Lepob), and db/db (B6.Cg-<sup>m/+</sup> lepr<sup>db</sup>) mice and their littermates were purchased from The Jackson Laboratory (Bar Harbor, ME). Male mice of 8 to 10 wk of age were used throughout the study. Mice were maintained with standard rodent chow and had free access to water.
Materials

LPS was purchased from List Biologic Laboratories (Campbell, CA). Recombinant mouse leptin was purchased from R&D Systems (Minneapolis, MN). Other chemicals were purchased from Sigma Chemical Co. (St. Louis, MO) unless otherwise specified.

Measurement of GFR and Mean Arterial Pressure

The animals were anesthetized with pentobarbital (60 mg/kg) and placed on a thermostatically controlled surgical table. A tracheotomy was performed, and a steady steam of 100% oxygen was blown over the tracheal tube throughout the experiment. Catheters (custom-pulled from PE-250 tubing) were placed in the jugular vein (for maintenance infusion) and the carotid artery (for BP determinations). Mean arterial pressure (MAP) was measured via a carotid artery catheter connected to a Transpac IV system (Datag Instruments). An intravenous maintenance infusion of 2.25% BSA in normal saline solution, at a rate of 0.25 μl/g body wt per min, was started 1 h before experimentation. FITC-inulin (0.75%) was added to the infusion solution for the determination of GFR, as described by Lorenz and Gruenstein (7). A bladder catheter (PE-10) was used to collect urine. Two 30-min collections of urine were obtained under oil and weighed for volume determination. Blood samples for plasma inulin determinations were obtained between urine collections. FITC levels in plasma and urine samples were measured with a CytoFluor plate reader (PerSeptive Biosystems).

Measurement of Serum Leptin Levels

Serum leptin levels were measured with a Quantikine M immunoassay kit (R&D Systems). Briefly, a leptin standard and serum samples were incubated for 2 h at room temperature in a plate coated with polyclonal antibody to mouse leptin. Antibody conjugated to horse-radish peroxidase was then added to the plate after washes. Finally, substrate solution was added and the OD was determined with a microplate reader at 450 nm.

Measurement of Blood Glucose Levels

Blood glucose levels were measured with an Accu-Chek Advantage system (Boehringer Mannheim, Indianapolis, IN).

Histologic Examinations

Kidneys that had been fixed in 4% paraformaldehyde and embedded in paraffin were sectioned at 4 μm and stained with hematoxylin and eosin, with standard methods. Histologic examinations were performed by a renal pathologist without knowledge of the intervention. Histologic changes attributable to tubular necrosis were quantitated by calculation of the percentage of tubules that displayed cellular necrosis, loss of the brush border, cast formation, and tubule dilation, as follows: 0, none; 1, 1 to 10%; 2, 11 to 25%; 3, 26 to 45%. At least 10 fields (magnification, ×200) were reviewed for each slide.

Results

Serum Leptin Levels during Endotoxemia in Mice

When C57BL/6 wild-type mice received intraperitoneal injections of LPS (2.5 mg/kg), leptin levels increased significantly, compared with the vehicle-treated control animals (21.6 ± 3.7 ng/ml versus 8.8 ± 1.7 ng/ml, P < 0.01) (Figure 1). In ob/ob mice, leptin levels were almost undetectable (1.3 ± 0.1 ng/ml) and there was no significant increase in response to LPS at 0.3 mg/kg (2.0 ± 0.3 ng/ml, P = NS versus baseline) (Figure 1). In comparison with both wild-type and ob/ob mice, db/db mice demonstrated higher baseline leptin levels (70 ± 4 ng/ml, P < 0.01 for both). The level increased significantly with LPS at 0.4 mg/kg (158 ± 23 ng/ml, P < 0.01 versus baseline) (Figure 1).

Renal Function in ob/ob and db/db Mice during Endotoxemia

When homozygous ob/ob mice received intraperitoneal injections of 0.3 mg/kg LPS, the GFR decreased significantly (0.37 ± 0.04 ml/min per g kidney versus 0.83 ± 0.06 ml/min per g kidney, n = 6, P < 0.01), whereas the MAP was unchanged (72.0 ± 1.6 mmHg versus 63.0 ± 6.0 mmHg, n = 4, P = NS) (Figure 2, A and B). Serum albumin levels (1.53 ± 0.07 g/dl versus 1.56 ± 0.09 g/dl, n = 5, P = NS) and hematocrit values (45.9 ± 1.5% versus 46.1 ± 1.5%, n = 5, P = NS) were comparable in control animals and LPS-treated ob/ob mice. Renal histologic examinations demonstrated changes indicating acute tubular injury in approximately 10% of tubules in ob/ob mice treated with LPS, compared with no tubular changes in littermates and db/db mice before and after LPS. In contrast, when ob/ob littermates (+/?ob) received injections of the same dose of LPS, there was no significant change in MAP (71 ± 3.0 mmHg versus 80 ± 3.4 mmHg, n = 4, P = NS) or GFR (0.77 ± 0.03 ml/min per g kidney versus 0.74 ± 0.04 ml/min per g kidney, n = 6, P = NS) at 16 h after LPS treatment (Figure 2, C and D). To examine whether obesity plays a role in the susceptibility to endotoxemic ARF, similarly obese db/db mice were used in the study. In contrast to ob/ob mice, there was no significant change in GFR (0.54 ± 0.04 ml/min per g kidney versus 0.65 ± 0.09 ml/min per g kidney, n = 4, P = NS) or MAP (88 ± 0.2 mmHg versus 87 ± 1.1 mmHg, n = 4, P = NS) when homozygous db/db mice received injections of an even higher dose of LPS (0.4 mg/kg) (Figure 3, A and B). There were no significant changes in GFR (0.57 ± 0.01 ml/min per g kidney versus 0.62 ± 0.12 ml/min per g kidney, n = 5, P = NS) and MAP (78 ± 0.5 mmHg versus
versus 80 ± 0.3 mmHg, n = 4, P = NS) when db/db littermates (db/+) received intraperitoneal injections of LPS at 0.4 mg/kg (Figure 3, C and D).

To further clarify whether leptin deficiency was responsible for the susceptibility to endotoxemic ARF in ob/ob mice, recombinant mouse leptin was administered to ob/ob mice before LPS administration. Vehicle or leptin (1 μg/g per d) was injected intraperitoneally for 10 d before mice were challenged with LPS (0.3 mg/kg, administered intraperitoneally). GFR were similar in the vehicle and leptin groups (0.52 ± 0.03 ml/min per g kidney versus 0.41 ± 0.02 ml/min per g kidney). MAP were also comparable in the two groups (93 ± 1 mmHg versus 83 ± 6 mmHg, P = NS). However, when mice were challenged with a higher dose of LPS (0.5 mg/kg), the mice in the vehicle-treated group became hypotensive and anuric. Serum creatinine levels were therefore used to assess renal function. Serum creatinine levels were significantly improved with leptin treatment, compared with vehicle treatment (0.4 ± 0.1 mg/dl versus 0.9 ± 0.1 mg/dl, P < 0.01). The MAP was significantly higher in the leptin group, compared with the vehicle group (68 ± 4 mmHg versus 51 ± 2 mmHg, n = 6, P < 0.01) (Figure 4).

Discussion

Increased serum leptin concentrations were observed after LPS administration in rodents (5,6), and leptin induction during inflammation was absent in IL-1β-deficient mice (8). The leptin-deficient ob/ob mice are more susceptible to LPS-related lethality than are their lean littermates (3). Although many of the cytokine responses to LPS were observed to be comparable in the ob/ob mice and their littermates, there was blunted

Blood Glucose Levels in ob/ob and db/db Mice

There was no significant difference in blood glucose levels between wild-type mice and littermates of ob/ob or db/db mice (141 ± 6, 136 ± 4, and 154 ± 15 mg/dl, respectively; P = NS). However, the blood glucose levels were significantly higher in ob/ob and db/db mice (223 ± 26 and 442 ± 50 mg/dl, respectively; n = 8, P < 0.01 versus wild-type mice and/or their littermates). Blood glucose levels were significantly decreased in both ob/ob and db/db mice 16 h after LPS injection (88 ± 18 and 105 ± 9 mg/dl, respectively; both P < 0.01 versus the respective baseline values). Therefore, blood glucose levels were comparable for ob/ob and db/db mice 16 h after LPS exposure, when renal function was examined.

Figure 2. GFR (A and C) and mean arterial pressure (MAP) (B and D) in ob/ob mice (A and B) and their littermates (C and D) during endotoxemia. The functional study was performed 16 h after LPS injection. GFR was measured as FITC-inulin clearance, and MAP was measured via the carotid artery. Values are mean ± SEM. CON, control.
induction of IL-10 and IL-1 receptor antagonist expression (3). The \( \text{db} / \text{db} \) mice, which are deficient in the Ob/Rb isoform of the leptin receptor, are as obese as \( \text{ob} / \text{ob} \) mice but are less susceptible to LPS lethality (3). The \( \text{db} / \text{db} \) mice exhibit high basal serum leptin levels, which increase with LPS exposure.

Because ARF is known to dramatically increase mortality rates among human subjects during sepsis (2), this study was undertaken to investigate whether there was a difference in the occurrence of ARF during LPS exposure for \( \text{ob} / \text{ob} \) versus \( \text{db} / \text{db} \) mice. The \( \text{ob} / \text{ob} \) mice were much more sensitive to LPS-related ARF than were their lean littermates. It was previously demonstrated, in a normotensive mouse model of LPS-related ARF, that wild-type mice must be exposed to 2.0 mg/kg (administered intraperitoneally) for a decrease in GFR of \( >50\% \) to occur 16 h after LPS (9). In contrast, the \( \text{ob} / \text{ob} \) mice demonstrated a similar decrease in GFR of approximately \( 50\% \) with only 0.3 mg/kg LPS. The conditions and the type of LPS were exactly the same for the wild-type and \( \text{ob} / \text{ob} \) mice. This increased renal functional sensitivity to LPS was not observed with even a slightly higher dose (0.4 mg/kg) of LPS in \( \text{db} / \text{db} \) mice. Only the \( \text{ob} / \text{ob} \) mice exhibited evidence of tubular necrosis after LPS treatment. The changes were mild, however, as most frequently observed in human ARF. There was no evidence that volume depletion was a factor in the ARF in the \( \text{ob} / \text{ob} \) mice during endotoxemia.

Although the \( \text{ob} / \text{ob} \) mice did not exhibit serum leptin levels at baseline or after LPS treatment, the \( \text{db} / \text{db} \) mice demonstrated significantly higher serum leptin levels at baseline and after LPS treatment, compared with wild-type mice. Leptin replacement for 10 d for the \( \text{ob} / \text{ob} \) mice did not afford protection with respect to the previously demonstrated decrease in GFR 16 h after 0.3 mg/kg LPS administration. The absence of protection with leptin was not associated with an effect on MAP. However, with 0.5 mg/kg LPS administration to \( \text{ob} / \text{ob} \) mice, endotoxemic shock was observed, with a MAP of approximately 50 mmHg. The mice were anuric and demonstrated significantly elevated serum creatinine concentrations. With the 10 d of leptin treatment, the \( \text{ob} / \text{ob} \) mice exhibited significantly higher MAP and significantly lower serum creatinine levels. Therefore, an effect of leptin to increase BP may be critical for the protection observed in the endotoxemic shock model of ARF. For septic patients, it has been demonstrated that plasma leptin levels are higher among survivors than nonsurvivors (10).

In summary, endotoxin-related ARF occurs at a much lower dose in leptin-deficient \( \text{ob} / \text{ob} \) mice than in their lean litter-

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\caption{GFR (A and C) and MAP (B and D) in \( \text{db} / \text{db} \) mice (A and B) and their littermates (C and D) during endotoxemia. The functional study was performed 16 h after LPS injection. GFR was measured as FITC-inulin clearance, and MAP was measured via the carotid artery. Values are mean ± SEM. CON, control.}
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The susceptibility to endotoxemia-related ARF in ob/ob mice cannot be exclusively attributable to obesity, however, because db/db mice, which are comparably obese, were resistant to a similar LPS dose. In the hypotensive endotoxemia-related model, ob/ob mice were protected against ARF with prior leptin treatment. The increased MAP with leptin treatment in the LPS shock model suggests an effect on peripheral vascular resistance. Such an effect is consistent with the demonstrated effect of leptin to stimulate the sympathetic nervous system (11,12). Whereas db/db mice lack the long isoforms of the hypothalamic leptin receptor, the short isoforms of leptin receptors are located in many tissues throughout the body, including the kidney and the vasculature (4). The increased levels of endogenous leptin acting on these short isoforms of leptin receptors in db/db mice may be critical for the observed renal protection against endotoxemia. Renal protection with exogenous leptin in the LPS-induced shock model in ob/ob mice may involve the same receptors. There are, however, other potential factors that may contribute to the increased sensitivity of ob/ob mice to endotoxemia-related ARF, such as blunted induction of anti-inflammatory cytokines. Finally, it should be emphasized that, although the occurrence of ARF may contribute to the increased mortality rates among endotoxemic ob/ob mice, deleterious effects on other organ systems may also be involved.

Acknowledgments
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References