Increased Leptin Expression in Mice with Bacterial Peritonitis is Partially Regulated by Tumor Necrosis Factor Alpha

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Increased Leptin Expression in Mice with Bacterial Peritonitis is Partially Regulated by Tumor Necrosis Factor Alpha

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Plasma leptin and *ob* gene mRNA levels were increased in mice following bacterial peritonitis, and blocking an endogenous tumor necrosis factor alpha (TNF-α) response blunted the increase. However, plasma leptin concentrations did not correlate with the associated anorexia. We conclude that leptin expression is under partial regulatory control of TNF-α in peritonitis, but the anorexia is not dependent on increased leptin production.

An important consequence of infection or sepsis is anorexia and a wasting of lean tissue. Proinflammatory cytokines initiate and modulate the host response to infection, and several cytokines, including tumor necrosis factor alpha (TNF-α), have been shown to induce anorexia and weight loss in healthy animals (2, 5). Endotoxin or TNF-α administration also increases leptin expression (3, 9), and leptin has been shown to act in an endocrine fashion to regulate food intake and body weight (11). In the present study, we investigated whether leptin expression was increased in mice suffering from bacterial peritonitis. In addition, we examined the role of endogenously produced TNF-α in regulating leptin expression in bacterial peritonitis by using a novel TNF inhibitor.

Female C57BL/6 mice weighing 17 to 23 g (Charles River, Wilmington, Mass.) were housed four per cage in a temperature-controlled room with a constant light-dark cycle (0700 to 2100 h). Beginning at 0700 h, mice were anesthetized with 100 mg of ketamine HCl (Fort Dodge Laboratories, Inc., Fort Dodge, Iowa) per kg, and 48 mice underwent a laparotomy. Cecal ligation and puncture were performed by ligating the base of the cecum with a 3-0 silk suture and creating two enterotomies midway between the tip and base of the cecum with a 22-gauge needle, as described previously (6). An additional 31 mice were pretreated with 5 mg of a TNF-binding protein (TNF-bp; Amgen, Boulder, Colo.) per kg of body weight intraperitoneally 30 min prior to cecal ligation and puncture. Twenty-five control mice underwent a sham operation, which was comprised of anesthesia, laparotomy, and mobilization of the bowels. Of these, 17 were pair fed quantities of food equivalent to that for the cecal-ligation and puncture group and 8 were freely fed.

Blood was obtained from the retroorbital plexus at intervals thereafter. Animals were sacrificed by cervical dislocation, and peritoneal lavage was performed by instilling 1.0 ml of 0.9% sodium chloride into the peritoneal cavity and aspirating the effluent. Inguinal fat pads were immediately removed for RNA isolation. Plasma leptin levels were determined with a commercial radioimmunoassay (Linco, St. Charles, Mo.). Plasma and peritoneal-lavage effluent TNF bioactivities were measured by a WEHI 164 clone 13 cytotoxicity assay, as previously described (10). Plasma and peritoneal-lavage interleukin 6 (IL-6) levels were measured by an enzyme-linked immunosorbent assay with commercially available antibodies (Endogen, Cambridge, Mass.).

Total RNA was extracted from the fresh adipose tissue samples by the acid-phenol and guanidinium isothiocyanate method (1), and 25 μg of total cellular RNA per lane was electrophoresed in a 1% agarose-formaldehyde gel, transferred to a Zetabind (Cuno, Inc., Meriden, Conn.) nylon membrane, and cross-linked by ultraviolet exposure. Partial murine *ob* gene cDNA (provided by Satya Kalra, University of Florida College of Medicine) and complete Cu-Zn super oxide dismutase cDNA (provided by Harry Nick, University of Florida College of Medicine) were labeled with [^{32}P]dATP by utilizing a commercial random primer extension kit (Stratagene, Inc., La Jolla, Calif.) and used for hybridization and autoradiography by applying standard techniques. Autoradiographs were scanned with a flatbed scanner, and the intensities of the signals were determined with a commercial package (SigmaScan; Jandel Scientific, Santa Clara, Calif.).

Following cecal ligation and puncture, plasma leptin levels

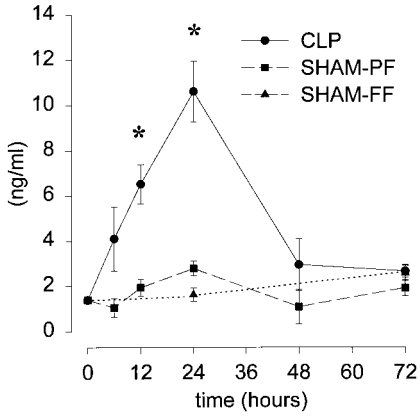

FIG. 1. Plasma leptin levels after cecal ligation and puncture. Plasma leptin levels after cecal ligation and puncture (CLP) were significantly elevated at 12 and 24 h over the values at time zero and were significantly higher than in freely fed, sham-treated mice (SHAM-FF) and in sham-treated mice pair fed quantities of food equivalent to that for the cecal-ligation and puncture group (SHAM-PF). The asterisks denote a P value of <0.05.

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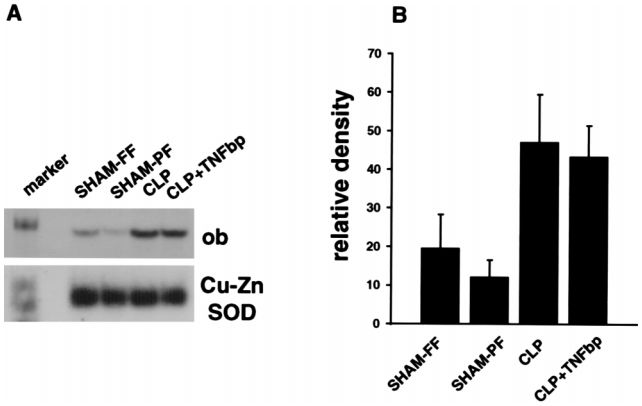

FIG. 2. Adipose tissue *ob* mRNA expression after cecal ligation and puncture with and without TNF-binding protein pretreatment. (A) Northern blot analysis of pooled RNA samples shows elevated *ob* mRNA expression 24 h after cecal ligation and puncture (CLP) and after cecal ligation and puncture with pretreatment with TNF-bp (CLP+TNFbp), relative to levels in freely fed sham-treated (SHAM-FF) and pair-fed, sham-treated (SHAM-PF) mice. Cu-Zn super oxide dismutase (SOD) mRNA levels were used to confirm equivalent loading of RNA. (B) Densitometric analysis of *ob* mRNA levels from five random samples of RNA in each group. *ob* mRNA expression was significantly increased after cecal ligation and puncture, compared to levels in pair-fed and freely fed, sham-treated animals. In contrast, there was only a modest attenuation of this *ob* mRNA in the TNF-bp-pretreated group.

significantly increased, peaking at 24 h and declining to baseline thereafter (Fig. 1). The relative increases in plasma leptin concentration were significantly different ($P < 0.05$) compared to baseline measurements (by one-way analysis of variance and post hoc analysis) and to results for pair-fed and freely fed, sham-treated animals (by two-way analysis of variance and post hoc analysis) at similar time points. Similarly, adipose tissue *ob* mRNA levels were examined when plasma concentrations peaked (at 24 h) and were significantly higher in septic mice, compared to results for pair-fed and freely fed controls (Fig. 2).

TNF bioactivity was only episodically detected (>10 pg/ml) in the plasma and peritoneal-lavage fluid of mice after cecal ligation and puncture (Table 1). Pretreating mice with TNF-bp prior to bacterial peritonitis inhibited plasma and peritoneal TNF activity at 24 h ($P < 0.05$). In contrast, plasma and peritoneal-lavage IL-6 levels were not significantly different for septic animals pretreated and those not pretreated with TNF-bp (Table 1). However, inhibiting TNF activity significantly reduced peak plasma leptin levels 24 h after cecal ligation and puncture, from 10.6 ± 1.3 ng/ml to 6.2 ± 1.0 ng/ml ($P < 0.05$). In contrast, *ob* mRNA levels were only modestly decreased with TNF inhibition (Fig. 2), suggesting that TNF-α acts at least in part posttranscriptionally to regulate leptin levels.

Food intake was reduced similarly over the first 72 h in sham-treated mice and in mice with a cecal ligation and puncture (Table 1). Similarly, pretreatment of cecal-ligation and puncture mice with TNF-bp had no effect on food intake, despite differences in plasma leptin response. Mortality rates at 24 h after cecal ligation and puncture were nearly identical in the TNF-bp-pretreated (19%) and untreated groups (20%).

Although leptin has been shown to play an important physiological role in regulating food intake in normal and obese animals (8), its role in regulating food intake during infection is much less clear. Sarraf and Grunfeld suggest that during acute inflammation, proinflammatory cytokines such as TNF-α may induce anorexia and weight loss via induction of leptin (3, 9). Our results are only partially supportive of this hypothesis. We can confirm that lean mice have a transient increase in the plasma leptin response to bacterial peritonitis and that inhibition of TNF activity attenuates this response. The absence of any difference in either mortality or IL-6 levels between septic mice with and without TNF-bp suggests that the inhibition of leptin production may be a specific response to TNF blockade, rather than to any overall reduction in the severity of the systemic inflammatory response.

Despite this increase in leptin response to bacterial peritonitis, the data do not support a role for leptin as the central mediator of early anorexia in this model. The stress of anesthesia and laparotomy was sufficient to induce a significant degree of anorexia but not a plasma leptin response in sham-treated mice, and TNF blockade attenuated the leptin response but not the anorexia.

TABLE 1. Food intake and plasma and peritoneal cytokine responses

Time elapsed (h)	Group[a]	TNF-α (pg/ml) in:				IL-6 (pg/ml) in:[c]		Food intake (g/day)[d]
		Plasma		Peritoneal lavage fluid		Plasma	Peritoneal lavage fluid	
		No. of responses[b]	Median (range)	No. of responses[b]	Median (range)			
0	Sham-PF	0/2	0	0/2	0	0	0	3.62 ± 0.16
	CLP	0/3	0	0/3	0	0	0	3.95 ± 0.13
	CLP plus TNF-bp	0/3	0	0/3	0	0	101 ± 101	3.93 ± 0.17
8	Sham-PF	0/2	0	0/3	0	11,999 ± 11,370	47,958 ± 46,871	ND
	CLP	5/9	159 (0–620)	1/9	0 (0–35)	96,635 ± 36,883	401,081 ± 83,080	ND
	CLP plus TNF-bp	3/6	0 (0–157)	0/6	0	89,695 ± 33,659	208,061 ± 65,141	ND
24	Sham-PF	0/2	0	0/2	0	331 ± 41	355 ± 117	0.26 ± 0.12
	CLP	9/11*	65 (0–620)	4/11	0 (0–601)	277,266 ± 133,563	141,989 ± 77,524	0
	CLP plus TNF-bp	3/19	0 (0–63)	1/19	0 (0–46)	209,540 ± 109,477	123,719 ± 40,912	0

[a] Sham-PF, sham-treated mice pair fed quantities of food equivalent to that for mice given cecal ligation and puncture; CLP, mice give cecal ligation and puncture; CLP plus TNF-bp, mice given cecal ligation and puncture and treated with TNF-bp.

[b] Number positive/total number. The asterisk indicates a P value of <0.05 in comparison to the group given cecal ligation and puncture plus TNF-bp, by Fisher's exact test.

[c] Mean ± standard error of the mean.

[d] Food intake is given as the mean food (± standard error of the mean) consumed in the 24-h period immediately prior to the cecal ligation and puncture or sham procedure. ND, not done.

If leptin is not involved or contributes only modestly to anorexia in this model, what mediators are contributory? In previous studies with turpentine-induced myositis, we and others have shown that endogenous production of IL-6 contributes significantly to the anorexia and loss of body weight that occur (4, 7). IL-6 does not induce leptin expression (9) but signals through a similar gp130-like pathway, suggesting that the mediators which regulate food intake in health and in disease may be profoundly different.

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