

RECOMBINANT HUMAN TUMOR NECROSIS FACTOR
INDUCES ACUTE REDUCTIONS IN FOOD INTAKE AND
BODY WEIGHT IN MICE

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Cachexia, a frequent pathophysiological complication of malignancy, is characterized by progressive wasting of adipose and muscle tissues, anorexia, asthenia, and anemia (1-3). In a portion of cancer patients, wasting can be accounted for by neoplastic invasion of the gastrointestinal tract or by toxic injury resulting from chemotherapy. However, in most cases, the development of cachexia cannot be correlated with tumor burden, pathology, anatomic location, or treatment. Alterations in lipid, protein, and carbohydrate metabolism observed in cancer cachexia (1, 2) are similar to those detected in major injury or sepsis (4, 5).

Recent attention has focused on the role of TNF/cachectin in the development of cachexia in neoplastic and infectious disease (6). TNF has numerous biological activities including suppression of lipoprotein lipase (LPL) activity in adipocytes (7). Reduced LPL activity in plasma and adipose tissue correlates with, and may be responsible for, the hyperlipidemia and weight loss that are often observed in cancer patients (8, 9). Data from two studies support the hypothesis that TNF induces weight loss in cancer cachexia. Treatment of mice with conditioned media from endotoxin-activated macrophages, which contains TNF, reduces food intake and weight (10). Recently, we reported that nude mice, with tumors that secrete TNF, develop cachexia (11).

We have extended those studies to test whether treatment with recombinant human TNF (rHuTNF) induces cachexia in normal mice. rHuTNF was administered by injection twice daily or by implantation of osmotic pumps. Reductions in food intake and body weight were induced initially by rHuTNF. However, with continued treatment, the mice developed a tolerance to rHuTNF and resumed their pretreatment appetite and weight.

Materials and Methods

Mice. Endotoxin-resistant C3H/HeJ female mice were obtained from the Jackson Laboratory, Bar Harbor, ME. Mice weighed 19-23 g and were 8-14 wk old at the time of treatment. Mice were housed six per cage and fed ad libitum. Food intake and body weight were measured daily.

HuTNF Purification. rHuTNF, purified from *Escherichia coli* bearing a plasmid with an

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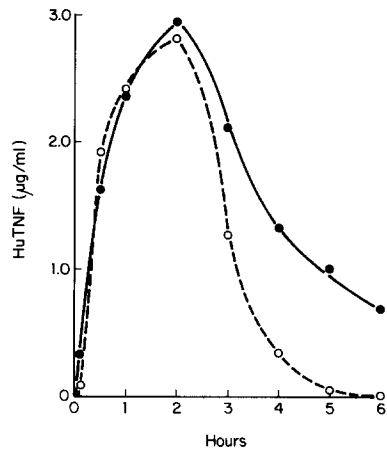


FIGURE 1. Clearance of rHuTNF from serum. Mice were injected intraperitoneally with 25 µg ^{125}I -rHuTNF and sera collected at the indicated times. The serum levels of rHuTNF were quantitated by radioactivity (●) and by the radio-receptor assay (○).

HuTNF gene (12), was >90% pure by SDS-PAGE and had a specific activity of $1-5 \times 10^7$ cytolytic U/mg.

Treatment of Mice with rHuTNF. rHuTNF was administered intraperitoneally by either injection or implantation of osmotic pumps. rHuTNF was injected in 0.5 ml of PBS containing 0.1% BSA. Alzet mini-osmotic pumps (model 2002; Alza Corp., Palo Alto, CA) were filled with rHuTNF in 0.85% NaCl containing 0.1% BSA; the pumps delivered rHuTNF at a rate of 0.5 µl/h. When tested by the Limulus amoebocyte lysate assay, rHuTNF at 50 µg/ml in the injection vehicle, rHuTNF at 1 mg/ml in the pump vehicle, the injection vehicle alone, and the pump vehicle alone contained 30.72, 31.13, 30.72, and 29.76 endotoxin U/ml, respectively.

Iodination of rHuTNF. ^{125}I -rHuTNF, radiolabeled by a solid phase lactoperoxidase procedure (13), had an initial specific activity of 60-200 µCi/µg and retained >90% of the cytolytic activity of unlabeled rHuTNF.

Serum Levels of rHuTNF. Mice were injected intraperitoneally with 25 µg of ^{125}I -rHuTNF that had a specific activity of ~ 2 µCi/µg. Groups of three mice were killed and bled at designated times. rHuTNF in sera was quantitated by counting in a gamma counter and by a radioreceptor assay (14). Serum was also collected from four mice 24 h after placement of osmotic pumps that released 12 µg/day of rHuTNF. rHuTNF in those sera was measured by a sandwich ELISA (11) and by the radioreceptor assay.

Results

After intraperitoneal injection of mice with rHuTNF, serum levels of radiolabeled rHuTNF rose quickly, peaked at 3 µg/ml at 2 h, and dropped to 0.69 µg/ml within 6 h (Fig. 1). When serum rHuTNF was quantitated by the radioreceptor assay, similar kinetics were observed, except that the level of biologically active rHuTNF was <50 ng/ml 5 h after injection. Similar results were reported for the clearance of native mouse TNF from the serum of CD-1 mice after intraperitoneal injection (15). Serum levels of rHuTNF were 72-148 ng/ml 24 h after placement of pumps that released 12 µg rHuTNF/day.

We next looked at the effects of rHuTNF on appetite and body weight. Injections of rHuTNF twice a day caused a greater reduction in food intake than the same total dose given as a single injection (data not shown). Next, we tested whether repeated twice daily intraperitoneal injections of rHuTNF would result in sustained reductions in appetite and weight. Dose-dependent decreases in food intake and weight

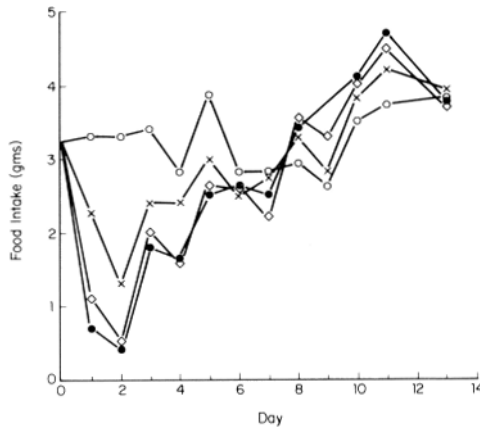


FIGURE 2. Effects of rHuTNF on food intake. Average food intake of mice (6/group) injected intraperitoneally twice a day with: PBS containing 0.1% BSA, vehicle control (O); 3 µg rHuTNF (x); 12 µg rHuTNF (◇); 24 µg rHuTNF (●).

were observed initially (Figs. 2 and 3). Mice treated with 24 µg rHuTNF/injection lost 17% of their pretreatment weight after 4 d of treatment. However, after the initial reduction, mice increased their food consumption and regained their pretreatment weight in spite of continued twice daily injections of rHuTNF. Thus, the mice developed a tolerance to rHuTNF. Doses of rHuTNF >25 µg twice a day were lethal.

The reductions in food intake and weight observed after treatment with rHuTNF do not appear to be due to contaminating LPS in the rHuTNF preparations. Similar levels of endotoxin, i.e., ~30 U/ml, were found in the rHuTNF/vehicle and vehicle preparations. Injection of 10 µg LPS (~1,000 endotoxin units) twice a day did not reduce food intake and weight of endotoxin-resistant mice (data not shown). Moreover, injection of 10 µg LPS with 20 µg rHuTNF twice a day did not enhance the reductions in appetite and weight observed after treatment with rHuTNF alone.

We questioned whether TNF must be present continuously to induce a sustained weight loss. Continuous intraperitoneal administration of rHuTNF was achieved by implantation of mini-osmotic pumps. Dose-dependent, biphasic reductions in food intake (data not shown) and weight (Fig. 4) were observed in mice bearing pumps

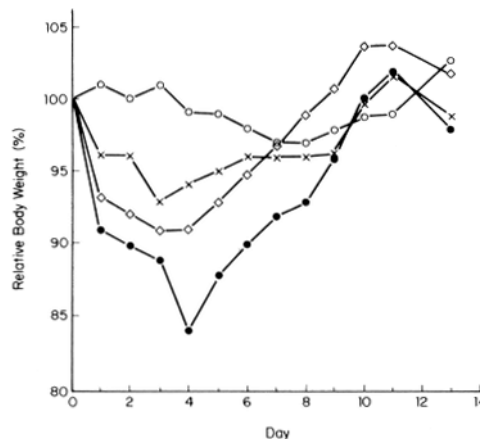


FIGURE 3. Effects of rHuTNF injected intraperitoneally on body weight. Relative changes in body weight of mice (6/group) treated twice daily with: PBS containing 0.1% BSA, vehicle control (O); 3 µg rHuTNF (x); 12 µg rHuTNF (◇); 24 µg rHuTNF (●).

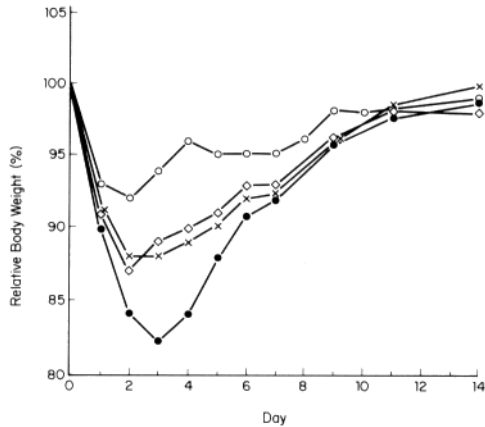


FIGURE 4. Effects of rHuTNF administered by intraperitoneally implanted pumps on body weight. Relative changes in body weight of mice (6/group) bearing osmotic mini-pumps releasing: 0.85% NaCl containing 0.1% BSA, vehicle control (O); 0.75 µg rHuTNF/day (x); 3.0 µg rHuTNF/day (◇); 12 µg rHuTNF/day (●).

that released rHuTNF. The time course of weight loss and regain was similar to that observed after repeated injections of rHuTNF. Mice that received higher doses of rHuTNF, i.e., ≥ 25 µg/day, died within 3 d of placement of the pumps.

Since treatment with a constant daily intraperitoneal dose of rHuTNF failed to induce long-term weight loss, we examined the effects of escalating doses of rHuTNF. Mice were injected intraperitoneally twice daily with rHuTNF at 12.5 µg/injection on days 0, 1, and 2; at 18.75 µg/injection on day 3; at 25 µg/injection on days 4 and 5; at 37.5 µg/injection on day 6; and at 50 µg/injection on day 7. Even with an increase to 50 µg rHuTNF twice a day, the mice regained their pretreatment appetite and weight.

The observed tolerance to rHuTNF faded within <3 wk of the termination of treatment. 17 d after the last injection of rHuTNF in the escalating dose experiment described above, the mice were treated twice a day for 2 d with 25 µg rHuTNF. Comparable reductions in food intake and weight were measured in mice that had shown tolerance to rHuTNF earlier and in mice that had not been previously treated with rHuTNF.

Discussion

Intraperitoneal administration of rHuTNF induced only short-term reductions in food consumption and weight in endotoxin-resistant mice in spite of continued treatment with rHuTNF. The mechanism by which rHuTNF causes appetite suppression and weight loss is not known. Semb et al. (16) found that a single intraperitoneal dose of 30 µg of recombinant murine TNF suppressed adipose LPL for at least 48 h. A similar dose of rHuTNF in our hands had a small effect on food intake and no measurable effect on weight. It seems unlikely that suppression of adipose LPL alone is responsible for the weight loss observed here after multiple injections of rHuTNF. Remick et al. (17) have shown that a single intravenous injection of TNF had cytopathological effects on the gastrointestinal tract including development of a vascular leak syndrome and damage of villi and endothelial cells. Such damage to the gastrointestinal tract may account for the suppression and weight loss observed after treatment of mice with rHuTNF.

The mechanisms that underlie the induction of tolerance to rHuTNF are not defined.

Several observations indicate it is unlikely that immunological factors contribute to its development. A similar transitory response was observed when mice were treated with native mouse TNF (10), which is not likely to be immunogenic in mice. We detected low levels of antibodies against rHuTNF in only a few mice that developed resistance to rHuTNF. Also, transitory decreases in food intake and weight were observed when nude mice were treated with rHuTNF (data not shown). Additionally, the resistance developed rapidly and faded in <3 wk, which is uncharacteristic of immune-mediated resistance. Early-phase tolerance to endotoxin, which induces TNF production in vivo (6) and in vitro (7), develops within a few days of treatment, but fades within a week (18). Similarities exist between early-phase tolerance to endotoxin and tolerance to rHuTNF.

We reported that chronic appetite suppression and weight loss occurred in nude mice with tumors that secrete rHuTNF (11). That observation contrasts sharply with the biphasic decreases in food intake and weight in normal mice treated with rHuTNF reported here. Yet, higher levels of rHuTNF were detected in sera from normal mice treated with rHuTNF, i.e., almost 3 µg/ml after injection and up to 148 ng/ml in mice with pumps, compared with 1-23 ng/ml in the tumor-bearing mice. The ability of rHuTNF to induce anorexia and cachexia may be dependent on the physiologic/disease status of an animal, and not simply a function of TNF dose. It is well documented that neoplastic disease produces a wide range of effects in multiple organ systems. Moreover, Bartholeyns et al. (19) recently reported that mice bearing selected solid tumors showed increased sensitivity to the lethal effects of endotoxin and TNF. Resolution of the apparent dichotomy in the ability of TNF to induce weight loss in normal and tumor-bearing mice should help define the role of TNF in the development of cancer-associated cachexia.

Summary

We examined the effects of treatment with rHuTNF on food consumption and body weight in C3H/HeJ mice. rHuTNF was administered intraperitoneally either by injections of 3, 12, or 24 µg twice a day or by implantation of osmotic pumps that released 0.75, 3, or 12 µg per day. Dose-dependent reductions in both food intake and weight were induced by rHuTNF. However, in spite of continued exposure to rHuTNF, the mice developed a resistance to rHuTNF and resumed their pretreatment food intake and weight. Non-immunological factors may play a role in the development of this tolerance, since it developed rapidly and faded within 2 wk of cessation of exposure to rHuTNF.

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