Cachectic Effects of Recombinant Human Tumor Necrosis Factor in Rats

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ABSTRACT

Treatment of rats with either intermittent bolus i.v. injections or continuous i.v. infusions of the same sublethal daily dose of tumor necrosis factor (TNF) results in decreased food intake and decreased nitrogen balance compared to saline-treated control rats. After 4 days of treatment, rats treated with intermittent bolus doses of TNF develop tolerance to the nutritional effects and consume normal amounts of food and have nitrogen balance similar to those of saline treated rats. Rats receiving the continuous infusion of TNF do not. Rats treated with both routes of TNF lose more weight than pair fed rats who eat the same mean amount as the continuous TNF treated group. In addition, 56% of rats receiving continuous infusion TNF die during the 8-day experimental period while rats receiving either intermittent bolus TNF or similar food intake (pair fed) do not. Body composition studies of rats that completed the 8 days of treatment indicate that rats receiving either continuous infusion or intermittent bolus TNF have increased percentages of body water and reduced percentages of body solid compared to saline treated control rats. Rats pair fed to the food intake of continuous TNF treated rats also had increased percentages of body water and reduced percentages of body solid, but changes were significantly less than those observed in continuous TNF infused rats. Continuous TNF infusion reduced total body nitrogen and potassium while pair feeding did not reduce potassium and reduced nitrogen to a lesser degree. Pair feeding and continuous TNF infusion reduced total body fat to a similar extent. Twice a day administration of TNF resulted in lesser changes in carcass water, solid, nitrogen, lipid, and potassium than continuous infusion of the same dose of TNF.

The results indicate that continuous infusion of TNF can produce anorexia, weight loss, edema, loss of body protein, lipid and cell mass, and lethality which is markedly ameliorated with bolus doses of TNF. The findings are consistent with the hypothesis that slow continuous secretion of sublethal amounts of TNF may mediate cancer cachexia.

INTRODUCTION

Cachexia associated with cancer and chronic inflammatory conditions is a complex syndrome characterized by declining food intake (anorexia) and altered metabolism which if unabated will ultimately result in death (1-3). The host alterations are composed of a loss of lipid and protein mass, a gain of total body water, asthenia, and anemia greater than that due to simple starvation alone (4, 5). The cachexia of chronic inflammatory conditions such as parasitic infections or infected burn wounds is clearly associated with excessive cachectin/tumor necrosis factor production (6-9), and some of the metabolic alterations associated with these conditions such as hypertriglyceridemia and decreased lipoprotein lipase activity are reproducible by TNF both in vivo and in vitro (6, 10).

Similarly, the cachexia of malignancy has been theoretically (1) and experimentally (11, 12) associated with circulating factors, and TNF has been implicated as one of these factors (13). However, the available evidence that TNF is a mediator of cancer cachexia is contradictory and inconclusive. One study (14) reported that a high proportion of cancer patients have circulating levels of TNF, but other studies have not been able to measure circulating levels of TNF in patients with advanced malignancies (7, 8). No study has demonstrated a correlation between circulating levels of TNF and clinical parameters of cachexia.

Whether the chronic administration of recombinant TNF is able to reproduce the anorexia and metabolic alterations of cancer cachexia is also unclear. rhTNF when administered to either rats or mice causes reduced food intake and weight loss (15-20). However, several groups independently have described tolerance to this anorectic effect; i.e., after repeated exposure to rhTNF rodents can eat normal amounts and gain weight despite continued administration of rhTNF (16-20). The observed tolerance to the nutritional effects and failure to demonstrate persistent anorexia with chronic administration of TNF may be due to the effects of a human protein in a rodent as suggested by some (21), but other evidence suggests that it may be due to intermittent rather than continuous administration. One study in which TNF was given by continuous i.v. infusion resulted in a marked decline in food intake without any change in the anorectic effect (15). In another study, in which the human TNF gene was cloned into a mammalian expression vector and transfected into a tumorigenic rodent cell line, tumor cell clones secreted TNF continuously and animals developed severe progressive anorexia and ultimately died of apparent cachexia (22). However, there is even controversy about this point because there is some evidence that mice can become resistant to the nutritional effects of a constant infusion of recombinant human TNF (19).

Finally, evidence that TNF causes a loss of total body fat or protein mass and a gain of total body water content is conflicting. Tracey et al. (17) were able to demonstrate a TNF-induced loss of body protein and body fat compared to both normal and pair fed controls, but they used escalating doses of rhTNF in order to overcome nutritional tolerance. One study demonstrated a loss of body fat (22), while several other studies did not detect any change in body protein (15, 22). In addition, body water did not change in one study (17) and it increased in another (15).

In an attempt to clarify whether tolerance to the nutritional and lethal effects of recombinant human TNF in rodents is related to method of administration and whether chronic administration of TNF can induce nutritional changes consistent with cachexia, we evaluated the chronic administration of either intermittent or continuous i.v. rhTNF on survival, food intake, nitrogen balance, and body composition of rats.

MATERIALS AND METHODS

Animals and Infusion Apparatus. Male Fischer 344 rats weighing between 200 and 280 g were used in each experiment. Rats were individually housed in metabolic cages that allowed daily collection of urine and stool. The room was lighted by a 12-h light/dark cycle. To allow accurate measurement of food intake, animals were fed C-21 ICN, Cleveland, OH), a nonscatterable semisynthetic paste diet that is nutritionally complete and has a nitrogen content of 40.6 mg nitrogen/g (20).

Rats were accustomed to the metabolic cages and diet for 3 days and...
then were anesthetized (pentobarbital, 35 mg/kg i.p.); and jugular catheters were placed using sterile technique (23). Catheters were attached to a spring and swivel mechanism to allow venous access and unrestrained movement. Normal saline was infused via Harvard pump at a rate of 1 ml/h. Rats were allowed free access to food and water.

After placement of the jugular catheters, animals were allowed to recover for 3 days and on the fourth postoperative day were entered into one of four experimental groups. By this time, rats had resumed normal food intake (see Fig. 3). Any animal eating less than 10 g/day was not entered into the study.

Reagents. Recombinant human tumor necrosis factor, the generous gift of Cetus Corporation, Emeryville, CA, had a specific activity of \(10^7\) units/mg by L929 cytoytic bioassay and an endotoxin level of 30 pg/2.5 x \(10^5\) units as measured by the Limulus assay (20). rhTNF was prepared each week by reconstituting the lyophilized powder under sterile conditions using PBS with 0.1% HSA. Aliquots for each day were then frozen at -20°C.

Radioimmunoassay (RIA) for hTNF. rhTNF was radiolabeled with \(^{125}\)I (New England Nuclear, Boston, MA) by a modification of the Iodo-Gen method using a solid phase reagent (Iodobeads; Pierce Chemicals) (24). Free iodine was removed by gel filtration on a 20-cm Sephadex G-25 column followed by dialysis against PBS (Biofluids). Total activity of the radiolabeled TNF was 4 x \(10^7\) cpm/ml and bioactivity was 1.64 x \(10^6\) units/ml by L929 assay (60% recovery of initial activity), giving a specific activity of 250 cpm/unit bioactivity. The RIA was performed by adding 50 \(\mu\)l of standard TNF or unknown sample to 250 \(\mu\)l of a 1:1500 dilution of rabbit anti-human TNF antibody (Endogen, Boston, MA) in PBS/0.2% bovine serum albumin (Grade V; Calbiochem at 4°C for 24 h, adding 100 \(\mu\)g cpm \(^{125}\)I TNF in 200 \(\mu\)l for 24 h at 4°C, adding goat anti-rabbit immunoglobulin (Immunobeads; Bio-Rad) for 12 h, centrifuging, and counting the pellet. The lower limit of detection in this assay was 150 pg/ml and the antibody did not cross-react with rodent TNF.

Infusion Studies. On the fourth post-jugular catheter insertion day, 26 rats were randomized into one of three groups: group a, normal saline (n = 9) that received a continuous infusion of 0.9% NaCl solution with 0.1% HSA; group b, twice daily TNF (BID TNF) (n = 8) that received a continuous infusion of 0.9% NaCl solution with 0.1% HSA and received 50 \(\mu\)g/kg rhTNF i.v. every 12 h; and group c, continuous TNF (n = 9) (CONT TNF) that received a continuous infusion of 0.9% NaCl solution with 0.1% HSA containing 100 \(\mu\)g/kg/day of rhTNF. There was a fourth group called PAIR FED (n = 9) that received a continuous infusion of 0.9% NaCl solution with 0.1% HSA and on day 4 post-catheter insertion was pair fed to the mean daily food intake of rats that received CONT TNF.

Fluids were administered i.v. by Harvard pump and each animal received 1 ml of fluid/h. In addition, i.v. lines were flushed twice daily with 1.5 ml of heparinized saline (100 units/ml) and rats in groups a, c, and d received a twice daily i.v. injection of 1 ml of 0.9% NaCl solution with 0.1% HSA to control for the extra volume received by group b rats. Solutions were changed daily and kept cool during administration by freezer packs.

Infusions were continued for 8 days (or until the animal died, whichever came first), rats were then sacrificed, catheters were removed, and carcasses were weighed. The intestine was opened, emptied, and returned to the carcass. Carcasses were then frozen at -20°C for compositional analysis.

Nitrogen Balance Studies. Food intake was measured daily and nitrogen intake was calculated based on the nitrogen content of C-21. Urine was collected in a container with 1 ml of concentrated sulfuric acid. Urine nitrogen was quantitated by chemiluminescence (Antek, Instruments, Inc., Houston, TX). Aliquots of stool were analyzed for nitrogen in a similar fashion. Nitrogen determinations were done with an intra- and interassay variation of less than 2%.

Compositional Analysis. The carcass was partially thawed and the pelt was removed and frozen in liquid nitrogen at -180°C. The frozen pelt was then shattered into a powder and returned to the carcass which was then homogenized with an industrial meat grinder ( Hobart, Troy, OH) and a commercial food processor (Robert Coupe) to ensure homogeneity. Careful weights were obtained at each step and all tissue was meticulously retrieved. Distilled water was added to give the homogenate a liquid consistency, and aliquots were lyophilized to constant weight. The dry weight of the animal was calculated from the weight of the lyophilized tissue (25).

One g of dry lyophilized homogenate was solubilized in 19 g of concentrated sulfuric acid by constant shaking at 50°C for 48 h. Liquidified samples were then analyzed for nitrogen content by chemiluminescence (Antek), and sodium and potassium contents were analyzed by flame photometry (26). Lipid was also extracted and measured from 200 mg of dried carcass homogenate according to the procedure of Folch et al. (27). All metabolic assays were performed in a blinded fashion.

Data for weight change and compositional analysis were analyzed only on rats that completed the 8-day infusion period. Early deaths were excluded from this analysis. If an animal died on day 8 of the infusion, it was included. Rats in the pair fed group weighed significantly less than each other group on entry into the study (see Table 1). Since compositional analysis parameters (total nitrogen, lipid, sodium, and potassium) were expressed per total carcass, results for the pair fed group were increased by 16% to equal to least 1 SD less than the mean initial weight of the other rats.

Serum Levels of TNF. Serum levels of human TNF were measured in 12 rats that were different from the rats described previously but handled in an identical manner. Six rats received a single i.v. injection of 50 \(\mu\)g/kg of rhTNF and 6 rats received a constant infusion of 100 \(\mu\)g/kg/day of rhTNF. Rats were placed in metabolic cages and attached to an i.v. line and a swivel apparatus as described. Following the same recovery period, rats received either a single i.v. dose of rhTNF or initiation of a constant infusion of rhTNF at time 0. Blood samples were obtained by retroorbital puncture just prior to rhTNF administration and at 1, 2, 8, 24, and 48 h after either the single i.v. dose or the constant infusion. Serum was separated and assayed for hTNF by RIA.

Statistics. Data are presented as mean ± SEM and analyzed by Wilcoxon rank sum test. Survival curves are constructed by the Kaplan-Meier method and analyzed for significant differences by the Breslow modification of the Kruskal-Wallis test (28).

RESULTS

Serum Levels of TNF. Rats that were treated with an i.v. bolus of TNF (50 \(\mu\)g/kg) had serum levels of hTNF of 118 ± 7 ng/ml measured 1 h after the i.v. bolus (Fig. 1), and levels became undetectable at 8 h following an i.v. bolus of rhTNF (50 \(\mu\)g/kg). Six rats were given a continuous infusion of TNF at the same total dose per day (100 \(\mu\)g/kg) developed plateau serum levels of TNF (18.5 ± 4 ng/ml) at 8 h and serum levels remained at this level throughout the infusion (Fig. 1).

Survival. Continuous infusion of TNF resulted in a 56% mortality during the 8-day experimental period. Mortality in
the continuous TNF group was significantly greater than mortality in both the BID TNF group and the pair fed group ($P < 0.05$; Fig. 2). Control rats infused with normal saline had one late death during the experimental period apparently due to an embolus during heparin flushing of the catheter.

**Food Intake, Nitrogen Balance, and Weight Change.** The day prior to the initiation of the study (day 0) rats from each group consumed greater than 10 g of food (Fig. 3). Control rats infused with normal saline ate between 10 and 12 g of food daily. Rats given TNF by continuous infusion or intermittent injection ate significantly less than control rats for the initial 5 days of the experiment (Fig. 3). However, rats given intermittent boluses of TNF ate significantly more food than rats receiving continuous infusion of TNF from experimental days 3–8. In addition, on days 6–8 rats receiving i.v. boluses of TNF ate the same amount as control rats (Fig. 3). The cumulative food intake for the entire study period was greatest for the normal saline group, intermediate for the BID TNF group, and least for the CONT TNF group (Table 2). Pair fed rats (by definition) had food intake identical to that for rats that received continuous TNF.

Normal saline treated rats remained in positive nitrogen balance throughout the experimental period (Table 1). Rats that received BID TNF remained in less positive nitrogen balance than normal saline treated rats on days 1–4, but by day 5 these rats were not significantly different from saline treated controls and by day 2 these rats were significantly greater than rats treated with continuous TNF and pair fed rats (Table 1). Rats treated with continuous TNF and rats pair fed to the same food intake were both in negative nitrogen balance from day 2 and were not significantly different from each other (Table 1).

Body weight change demonstrated that no group of animals gained weight during the study period (Table 2). The normal saline group and the pair fed group lost the least amount of weight and were not different from each other. The BID and continuous TNF groups both lost significantly more weight during the study period than the pair fed or normal saline group. In addition, the continuous TNF group lost significantly more weight than the BID TNF group (Table 2).

**Body Composition.** Rats treated with either continuous or BID TNF had a significantly greater percentage of body water than control rats treated with normal saline. Rats treated with continuous infusion TNF had a greater percentage of body water than rats treated with either BID TNF or pair fed controls (Table 3). Pair fed rats had significantly more carcass water than normal saline rats. As expected, the converse of these findings was true for percentages of solid carcass (Table 3). Rats treated with continuous infusion TNF had the lowest total body nitrogen and potassium consistent with the greatest loss of body protein and cell mass (Table 3). Rats pair fed to the food intake of continuous TNF rats also had a significant loss of nitrogen compared to saline treated rats, but they did not lose as much nitrogen as continuous TNF treated rats nor did they lose body potassium. Carcass nitrogen or potassium was not reduced by intermittent bolus TNF. TNF treatment both intermittent and bolus as well as pair feeding caused a significant reduction in total body lipid that was significantly less than the lipid content of saline treated rats. The lipid content of pair fed rats was not different from continuous TNF treated rats but was less than BID TNF treated rats. Total body sodium was significantly increased in the TNF continuous group compared to the pair fed group and was not different in the saline, TNF BID, and TNF continuous groups. Carcass potassium was significantly decreased in the continuous TNF treated rats and was similar in all other groups including pair fed rats (Table 3).

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**Fig. 2.** Survival of rats from each of four experimental groups, BID TNF, PAIR FED, normal saline, and CONT TNF. Rats from CONT TNF group had reduced survival compared to rats from BID TNF and pair fed group ($P < 0.05$). All rats were included in this figure: $n = 9$ normal saline, $n = 8$ BID TNF, $n = 9$ CONT TNF, and $n = 9$ pair fed.

**Fig. 3.** Food intake of rats from each of three experimental groups: normal saline ($\circ$), BID TNF ($\bullet$), and CONT TNF ($\Delta$). * Greater than continuous TNF and pair fed, $P < 0.01$. † Greater than BID TNF. ‡ Greater than normal saline. § Greater than each other group, $P < 0.01$. ‡ Greater than continuous TNF and pair fed, $P < 0.001$. * Greater than continuous TNF and pair fed, $P < 0.01$. / NA, not available (too few animals to allow meaningful data). 

**Table 1 Daily nitrogen balance during study period (days 1–8)**

<table>
<thead>
<tr>
<th>Group</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal saline</td>
<td>254 ± 47†</td>
<td>278 ± 23†</td>
<td>214 ± 33†</td>
<td>288 ± 35†</td>
<td>220 ± 34†</td>
<td>229 ± 31†</td>
<td>213 ± 58†</td>
<td>249 ± 32†</td>
</tr>
<tr>
<td>BID TNF</td>
<td>24 ± 70</td>
<td>69 ± 39</td>
<td>135 ± 34</td>
<td>101 ± 49</td>
<td>170 ± 22</td>
<td>169 ± 22</td>
<td>202 ± 42</td>
<td>205 ± 34</td>
</tr>
<tr>
<td>CONT TNF</td>
<td>35 ± 23</td>
<td>−54 ± 41</td>
<td>−52 ± 22</td>
<td>−33 ± 20</td>
<td>−16 ± 42</td>
<td>−33 ± 22</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Pair fed</td>
<td>14 ± 7</td>
<td>−23 ± 8</td>
<td>−44 ± 7</td>
<td>−101 ± 8</td>
<td>−42 ± 10</td>
<td>−81 ± 8</td>
<td>−30 ± 11</td>
<td>−67 ± 9†</td>
</tr>
</tbody>
</table>

$n$, number of animals at start of the study. As animals die in some groups $n$ becomes smaller (Fig. 2 indicates when rats from each group died).

Mean ± SEM.

Greater than each other group, $P < 0.01$.

Greater than continuous TNF and pair fed, but not BID TNF, $P < 0.001$.

Greater than continuous TNF and pair fed, $P < 0.01$.

NA, not available (too few animals to allow meaningful data).

Less than all other groups, $P < 0.02$. 

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DISCUSSION

Cancer cachexia clearly impacts negatively on the duration and quality of life of patients who suffer cancer (29). It is so common in end-stage, progressive cancer that its true incidence is difficult to quantitate, but it has been estimated that two-thirds of people who die of cancer are cachectic at death (30). Data from the Eastern Cooperative Oncology Group indicate that over 50% of cancer patients have weight loss at the time of diagnosis, and that patients with weight loss have a reduced median survival compared to similarly treated patients without weight loss (31). The components of cancer cachexia can be broadly categorized as decreased food intake (anorexia) and a variety of perturbations in carbohydrate, lipid, protein, and energy metabolism that combine to lead to rapid host tissue wasting and ultimately the demise of the individual (reviewed in Refs. 2 and 3). The present study suggests that chronic continuous i.v. administration of TNF may produce the anorexia, lethality, and the accelerated host tissue wasting seen in natural cachexia while intermittent bolus injections of TNF at the same dose has less cachetic effects.

Human recombinant TNF given as a continuous infusion to rats resulted in a 75% decrease in food intake sustained over 8 days, whereas bolus administration of the same dose resulted in an initial decrease in food intake but a rapid recovery to control levels despite continued treatment. These results agree with previously published reports that repetitive bolus TNF treatment leads to anorectic tolerance (16–20) and a single previous report that continuous infusion of TNF does not (15).

However, this observation appears to disagree with one previous report by Socher et al. (19) that suggests that continuous infusion of TNF may result in nutritional tolerance in mice. One difference between the two studies was that in the study of Socher et al. implanted i.p. osmotic minipumps were used and in the present study constant i.v. infusion was used. Serum levels of TNF were quantitated at 24 h in the minipump study but not at 72–96 h when mice began to recover. It may be that the minipump failed to continuously administer TNF after a certain time point.

The mechanism of tolerance to the nutritional effects of TNF induced by intermittent bolus doses of TNF is unclear, but we have previously demonstrated that it is not antibody mediated or related to altered pharmacokinetics (20). Because equivalent amounts of identical TNF preparations produce sustained anorexia when given continuously but not when given intermittently, it appears that peak serum levels are necessary for the induction of tolerance whereas sustained low levels of TNF do not produce it (Fig. 1). In addition, because the exact same preparation of TNF can produce tolerance by one method of administration and not another it suggests that previous explanations of tolerance, as an artifact of either a xenogenic recombinant rats or endotoxin contamination, are incorrect (21, 32). We favor the mechanism of TNF receptor down-regulation. Peak serum levels of TNF may be sufficient to saturate TNF receptors and cause down-regulation, while lower serum levels may continuously stimulate TNF receptors. Direct evidence supporting or refuting this proposed mechanism has not been obtained.

The results of the current study are consistent with the hypothesis that TNF is a mediator of the anorexia associated with natural cachexia. Conditions such as malignancy or chronic infections may result in sustained continuous release of endogenous TNF that may cause reduced food intake. The mechanism whereby continuous infusion or secretion of TNF induces anorexia is unknown but probably involves its gastrointestinal and/or central nervous system effects. Previous investigations have shown that TNF can cause altered intestinal motility characterized by decreased gastric emptying (18), intestinal injury, and necrosis through local platelet-activating factor production (33), as well as anorexia due to direct hypothalamic stimulation (34).

As continuous infusions of TNF produce severe anorexia, normal homeostatic mechanisms to preserve host protein (at the expense of lipid) should occur (4). Any disruption of this adaptive metabolism by TNF can be separated from simple anorexia alone by comparing nitrogen balance and compositional analyses among the freely fed normal saline controls, continuous infusion TNF group, and pair fed rats. Normal saline control rats are in stable, daily positive nitrogen balance (Table 1) and have normal body nitrogen stores (Table 3). Starvation alone (pair fed rats) produces a negative nitrogen balance and loss of body nitrogen (Table 3). Continuous infusion of TNF results in a similar negative nitrogen balance but additional loss of body nitrogen, implying that TNF causes

### Table 2 Body weight, cumulative food intake, and body weight change

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Initial wt (g)</th>
<th>Cumulative food intake (g)</th>
<th>Final wt (g)</th>
<th>Wt change for 8 days (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal saline</td>
<td>257 ± 9*</td>
<td>80 ± 4*</td>
<td>256 ± 10</td>
<td>-1 ± 0.5*</td>
</tr>
<tr>
<td>TNF BID</td>
<td>266 ± 13</td>
<td>51 ± 6*</td>
<td>243 ± 6</td>
<td>-23 ± 4</td>
</tr>
<tr>
<td>TNF continuous</td>
<td>245 ± 8</td>
<td>18 ± 2</td>
<td>197 ± 13</td>
<td>-38 ± 8*</td>
</tr>
<tr>
<td>Pair fed to TNF</td>
<td>202 ± 3*</td>
<td>18</td>
<td>200 ± 3</td>
<td>-2 ± 0.5*</td>
</tr>
</tbody>
</table>

a) All animals are included.
b) Greater than BID and continuous TNF, P < 0.001.
c) Normal saline and pair fed are not different from each other but are less than other two groups and TNF continuous is greater than TNF BID P < 0.03.
d) Greater than continuous TNF, P < 0.001.
e) Less than each other group, P < 0.01.

### Table 3 Body composition of rats that completed the 8-day study period

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>n</th>
<th>Carcass water (%)</th>
<th>Carcass solid (%)</th>
<th>Total nitrogen (g)</th>
<th>Total lipid (g)</th>
<th>Total sodium (µmol)</th>
<th>Total potassium (µmol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal saline</td>
<td>8</td>
<td>62 ± 1*</td>
<td>38 ± 1</td>
<td>6.1 ± 0.4</td>
<td>41.3 ± 5.1</td>
<td>12.2 ± 0.8</td>
<td>12.8 ± 0.9</td>
</tr>
<tr>
<td>TNF BID</td>
<td>8</td>
<td>67 ± 1</td>
<td>33 ± 1</td>
<td>6.2 ± 0.4*</td>
<td>24.0 ± 2.7</td>
<td>12.5 ± 0.5</td>
<td>12.8 ± 0.5</td>
</tr>
<tr>
<td>TNF continuous</td>
<td>5*</td>
<td>75 ± 1</td>
<td>25 ± 1</td>
<td>4.6 ± 0.2</td>
<td>18.4 ± 2.5*</td>
<td>14.3 ± 1.0</td>
<td>7.3 ± 0.7*</td>
</tr>
<tr>
<td>Pair fed to TNF</td>
<td>9</td>
<td>68 ± 1*</td>
<td>32 ± 1*</td>
<td>5.2 ± 0.1</td>
<td>12.2 ± 1.2</td>
<td>11.7 ± 0.3*</td>
<td>13.4 ± 0.5</td>
</tr>
</tbody>
</table>

a) Mean ± SEM.
b) TNF BID is same as normal saline; otherwise each is different from each other P < 0.04.
c) One rat died on day 8 of the study period and is included.
d) TNF continuous is same as TNF BID and pair fed, each is < saline, and pair fed is < TNF BID, P < 0.01.
e) TNF continuous less than each other group, P = 0.002.
f) Pair fed had an initial weight less than each other group (see Table 1). Body composition results (total nitrogen, lipid, sodium, and potassium) were increased by 16% to adjust for weight difference of this group on entry into study.
g) Pair fed is same as TNF BID; otherwise each is different from each other, P < 0.01.
h) Pair fed is less than TNF continuous, P = 0.008.
additional protein wasting over that due to TNF induced anorexia. Bolus TNF treatment causes an initial decrease in nitrogen balance that normalizes and body nitrogen is preserved.

The observation that continuous infusion of exogenous TNF resulted in decreased whole body protein (nitrogen) is supported by others. A tracer kinetic study using [1-14C]leucine in rats demonstrated that animals exposed to sublethal doses of TNF had increasing muscle proteolysis similar to that observed in cancer (35). Another study reported a similar significant decrease in total body protein following chronic administration of escalating doses of TNF (17). Other investigators have shown that i.p. escalating sublethal doses of human recombinant cachectin/TNF for 7 days in rats causes accelerated peripheral (skeletal muscle) protein wasting while preserving liver protein content (36). Similar results were demonstrated following acute short-term infusions of TNF to human cancer patients in that TNF caused an increase in whole body protein metabolism and an increased efflux of amino acids from the forearm muscles (37). The mechanism of TNF induced accelerated muscle protein breakdown may be partially mediated through the hypothalamic-pituitary-adrenal axis. Infusions of TNF into humans cause elevated plasma cortisol levels (37) and infusions in rats cause increased adrenal weight and elevated plasma levels of adrenocorticotropic hormone (38). TNF infusions in rats resulted in decreased nitrogen balance and decreased levels of carcass nitrogen similar to corticosterone infusions (38) and the nitrogen changes seen with TNF were diminished in adrenalectomized rats given only maintenance doses of corticosterone (38).

Body lipid was reduced in animals that received TNF or were pair fed to the food intake of rats treated with continuous TNF. The reduction in body lipid with pair feeding was more than that seen with BID TNF but was not different from that seen with continuous TNF (Table 3). Since BID TNF rats consumed more food than continuous TNF or pair fed rats (Table 2), it appeared that food intake was the major determinant of carcass nitrogen, lipid, and not the known effect of TNF on the enzyme lipoprotein lipase. Recent in vitro studies using recombinant TNF show no effect of TNF on lipid biosynthetic enzymes (10), no effect on lipolysis (39), and variable effects on lipoprotein lipase (10, 40, 41) and are consistent with the present observations that TNF causes lipid depletion but not in excess of animals with similar food intake. This finding may be related to activity. Pair fed rats are very active while TNF infused rats are much less active.3 Differences in activity of different groups may impact on results of compositional analysis.

Our results show that TNF given by either bolus injection or continuous infusion results in increased total body water, and animals treated with continuous infusion TNF had the highest percentage of body water (Table 3). Pair fed rats had a modest increase in total body water as is expected with starvation (4). However, continuous infusion of TNF caused an additional increase in percentage body water more than that due to TNF induced anorexia alone (pair fed group) (Table 3). This result was in concordance with previous pathological studies that demonstrated marked organ edema with TNF infusion (42, 43).

Our study shows that the continuous infusion of a sublethal daily dose of TNF results in a 56% mortality by 8 days, whereas similarly anorectic (or pair fed) rats had no deaths (Fig. 2). As a reflection that the method of TNF administration led to tolerance, no rats given the same amount of TNF as a twice daily bolus died. In fact, these BID TNF tolerant rats had not only a 100% survival but also a lesser reduction in food intake (Fig. 3), more positive nitrogen balance (Table 1), and normal total body potassium and nitrogen (Table 3).

Is TNF an etiological factor in cancer cachexia? The inability to measure circulating TNF reliably in humans with cancer does not eliminate TNF as an etiological factor for cancer cachexia for two reasons: (a) local production and local effects or paracrine activity of TNF may occur without circulating levels of TNF (44); (b) phase I studies giving TNF as a continuous infusion to humans show undetectable or minimally detectable circulating levels of TNF despite systemic toxicity (45), implying that TNF may have toxic effects below detectable circulating levels.

The results of the present study and other published reports discussed indicate that TNF given as a continuous infusion can cause hypophagia, increased body water, lethality, and the wasting of host protein and lipid seen in cachexia. The cause of lethality in cachexia is thought to result from the depletion of necessary host tissue through a combination of hypophagia and inappropriately accelerated metabolism, especially catabolism. Our results demonstrate that TNF had lethal effects separate from its anorectic effects (no pair fed rats died) and it appeared to produce accelerated protein metabolism as evidenced by a significant reduction in compositional nitrogen and potassium compared to pair fed animals. This study suggests that a constant infusion of TNF can reproduce many of the changes seen in cancer cachexia: anorexia; weight loss; increased body water; reduced fat, protein, and body cell mass; as well as lethality. Intermittent twice daily administration of the same dose of TNF markedly lessens these catabolic effects. The findings are consistent with the hypothesis that slow continuous release of TNF may be responsible for part or many of the changes seen in cancer cachexia. Definitive demonstration of the role of TNF in cancer cachexia awaits further studies evaluating local production of TNF in cachectic animals and humans, and the effect of TNF blocking agents on cancer induced anorexia, survival, and response to antitumor therapy. Recent work with mice bearing tumors demonstrated anti-TNF antibody reversal of cachectic parameters further corroborating the present observations and supporting the role for TNF in cancer cachexia (46).

REFERENCES


Cachectic Effects of Recombinant Human Tumor Necrosis Factor in Rats


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