Tumor Necrosis Factor/Cachectin Decreases Catalase Activity of Rat Liver

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ABSTRACT

Tumor bearing animal model. An inflammatory response in cachexia, infection, and inflammation (1-6). Although many of the biochemical changes in cachexia and inflammation have been well defined, it remains to be determined which of these changes are induced directly or indirectly by TNF.

One of the earliest biochemical events in the cancer-bearing host is a marked decrease in the catalase activity of the liver and, to a lesser extent, of the kidney (7, 8). The decrease in activity is due to a decrease in enzyme protein. Nakahara et al. isolated a crude protein termed “toxohormone” from a number of human tumors which when injected into mice resulted in a marked decrease in liver catalase activity (7). More recently, Kampschmidt et al. noted that rats treated with a crude preparation of endotoxin lipopolysaccharide from Escherichia coli (about 6 mg/kg body weight) showed a 50% decrease in liver catalase activity. Since it is now known that endotoxin induces TNF production by macrophages (10, 11), that many tumors produce TNF (12), and that the host can make TNF in cachexia and inflammation (1-6), it remains to be determined which of these changes are induced directly or indirectly by TNF.

MATERIALS AND METHODS

Animals. Male Sprague-Dawley rats (160 ± 13 g) were maintained on a 12-h light, 12-h dark cycle and were provided with water and chow ad libitum.

Experimental. Rats were given injections i.p. of 50 or 100 mg/kg/day of human recombinant TNF-α for 5 days. The TNF was generously donated by Asahi Chemical Industry of America and contained a negligible amount of endotoxin as contaminant (2.33 × 10^5 units TNF/mg protein; 0.35 pg endotoxin/10^6 units TNF; equivalent to 0.001 pg endotoxin/μg of TNF). Six experiments were performed, each consisting of “experimental” rats given injections i.p. of TNF and “control” rats given injections of the vehicle only (10% glycerol for Experiments 1 and 2, or phosphate-buffered saline containing 0.1% serum bovine albumin for Experiments 3-6).

Results. Experiments 1, 2, and 3 included 4, 4, and 5 control rats, respectively, and 6, 6, and 8 experimental rats given injections of 50, 100, and 100 mg/kg/day of TNF, respectively, for 5 days. The total daily dose was divided into two equal doses which were injected at approximately 12-h intervals. All rats were fed ad libitum.

Experiments 4 and 5 were similar to 2 and 3, except that both control and experimental rats were fed ad libitum for 4 days and then fasted for 24 h on day 5. Experiment 4 included 4 control rats and 6 experimental rats given injections of 50 mg/kg/day twice daily. Experiment 5 included 6 control and 5 experimental rats and differed slightly from Experiment 4 in that the total daily dose of 100 mg/kg/day was divided into three equal doses of 33.3 μg each injected at approximately 8-h intervals.

Experiments 6 included 4 control rats fed ad libitum, 6 experimental rats given injections of 50 μg/kg/day twice daily for 5 days, and 6 control pair fed rats which were given daily the same amount of food postulate that TNF/cachectin is one of the factors responsible for this decrease in catalase activity.

In humans and the rat, catalase is found predominantly in the liver, kidney, and RBC. Within the liver and kidney, it is located mainly in the peroxisomes, while in RBC it is partly in the cytoplasm and partly bound to the cell membrane (14). Catalase is a tetramer of M, 240,000 in which each subunit contains a ferriprotoporphyrin as a prosthetic group. It is involved in two types of reaction, termed catalatic and peroxi-

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The abbreviation used are: TNF, tumor necrosis factor; CAT, catalatic activity of catalase; PER, peroxi-

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as was consumed by their TNF-injected partners on the previous day. On day 6 (or day 7 for the pair-fed controls), all rats were anesthetized with ether and sacrificed by guillotine decapitation. Liver and kidneys were excised, frozen on dry ice, and kept frozen at -70°C until homogenized. In Experiment 3, a portion of each liver was placed in 2.5% glutaraldehyde for electron microscopic examination. Prior to homogenization, the tissues were weighed and homogenized in a Potter-Elvehjem homogenizer in 10 volumes of cold 0.2 M phosphate buffer, pH 7.4, containing 2 mM ethylene glycol-bis-β-aminoethyl ether, 2 M EDTA, and 10% glycerol. The homogenates were centrifuged at 30,000 x g for 30 min and the supernatant fluids were divided into two aliquots, one for the determination of CAT and PER, and the other, to which concentrated mercaptoethanol was added to a final concentration of 0.1 M, for the determination of glucose-6-phosphate dehydrogenase and lactate dehydrogenase activities.

In Experiment 6 whole blood was also collected from all rats immediately following guillotine decapitation and the sera were separated by centrifugation and kept at -70°C until assayed.

Biochemical Analyses. Protein concentration in the supernatant fluids was determined by a biuret method adapted to the DACOS discrete analyzer (Coulter Electronics, Inc., Hialeah, FL 33010). G6PD (EC 1.1.1.49) activity was determined by measuring the rate of formation of NADPH spectrophotometrically at 340 nm as glucose-6-phosphate is oxidized to 6-phosphogluconic acid by NADP. LD (EC 1.1.1.27) activity was determined by measuring the rate of decrease in the absorbance of NADH at 340 nm as it is oxidized to NAD by pyruvate. The CAT activity of catalase (EC 1.1.1.16) was determined by following the decrease in the absorbance of hydrogen peroxide at 240 nm, as described by Aebi (15). The PER activity of catalase was determined with ethanol as hydrogen donor as described by Yasmineh and lacalatedehydrogenase activities.

Electron Microscopic Studies. Tissues were fixed in 2% glutaraldehyde, postfixed in 1% osmium tetroxide, dehydrated in a graded series of ethanol, and embedded in Maraglas resin. Thin sections were cut on a Reichert microtome (Warner-Lambert Technologies, Bloomington, MN), stained with lead and uranium, and examined in a Phillips 200T electron microscope (Phillips Electronic Instruments, Schaumburg, IL).

Statistical Analysis. All data were analyzed by the 2-tailed unpaired Student's t test.

RESULTS

Enzyme activities (in units or kilounits/g tissue wet weight) in the liver and kidney of normal rats are shown in Table 1 as means of the activities of the fed control rats in Experiments 1, 2, and 3. Liver CAT had by far the greatest activity (55 kilounits/g tissue wet weight) and was about 5-fold the activity in kidney (11 kilounits/g). The PER activity of liver and kidney were 190 and 50 units/g, respectively, and were 290- and 220-fold lower, respectively, than the corresponding CAT activities. G6PD activity was approximately the same in the two tissues (2 units/g) and LD activity was about twice greater in the liver (746 units/g) than in the kidney (309 units/g).

Table 1 also shows the mean enzyme activities of the 24-h fasted control rats in Experiments 4 and 5. Mean PER, CAT, and LD activities were not significantly different from those of the fed controls in both liver and kidney. Mean activity of kidney G6PD was not significantly different in the liver, but increased significantly in the kidney (19%; P = 0.001).

Enzyme activities of the control and TNF-treated rats were calculated in units per g of supernatant protein. Tables 2 and 3 list the enzyme activities in the liver and kidney, respectively.

Administration of the lower dose of TNF (50 μg/kg/day; Table 2, Experiment 1) did not significantly change the CAT and PER activities in the liver. Both activities were significantly

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<table>
<thead>
<tr>
<th>Liver</th>
<th>Kidney</th>
</tr>
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<tbody>
<tr>
<td>CAT</td>
<td>Fed</td>
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<tr>
<td>55 ± 10</td>
<td>61 ± 18</td>
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<tr>
<td>PER</td>
<td>190 ± 31</td>
</tr>
<tr>
<td>G6PD</td>
<td>1.62 ± 0.27</td>
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<tr>
<td>LD</td>
<td>746 ± 84</td>
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</table>

* The 13 fed control rats and 10 fasted control rats represent the total number of control rats in Experiments 1-3, and Experiments 4 and 5, respectively (see Table 2).

† CAT activity is in kilounits/g tissue wet weight.

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Table 2

<table>
<thead>
<tr>
<th>Experiment a</th>
<th>n</th>
<th>TNF dose b</th>
<th>CAT c</th>
<th>PER</th>
<th>G6PD</th>
<th>LD</th>
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<tr>
<td>1C</td>
<td>4</td>
<td>314 ± 16</td>
<td>1110 ± 97</td>
<td>9.6 ± 1.3</td>
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<tr>
<td>1T</td>
<td>6</td>
<td>295 ± 31</td>
<td>1010 ± 165</td>
<td>14.8 ± 2.8</td>
<td>4230 ± 634</td>
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<tr>
<td>2C</td>
<td>4</td>
<td>411 ± 41</td>
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<td>7.9 ± 1.6</td>
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<td>2T</td>
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<td>785 ± 116</td>
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<td>3C</td>
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<td>282 ± 14</td>
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<td>3T</td>
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<td>18.3 ± 4.2</td>
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<td>6</td>
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<td>15.2 ± 2.3</td>
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<td>1263 ± 177</td>
<td>8.9 ± 1.2</td>
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<td>5T  d</td>
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<td>778 ± 124</td>
<td>16.0 ± 4.0</td>
<td>3948 ± 214</td>
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</table>

a Control/TNF pairs with P values of 0.01 or less are italicized.

b Doses shown represent the total daily doses of TNF injected in μg/kg body weight (see "Materials and Methods" for further details).

c CAT activity is in kilounits.

d All rats in Experiments 4 and 5 were starved on day 5.

P = 0.02.

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Table 3

<table>
<thead>
<tr>
<th>Experiment a</th>
<th>n</th>
<th>TNF dose b</th>
<th>CAT c</th>
<th>PER</th>
<th>G6PD</th>
<th>LD</th>
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<tr>
<td>1C</td>
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<td>15.2 ± 4.2</td>
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<td>438 ± 95</td>
<td>16.4 ± 1.4</td>
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<td>356 ± 70</td>
<td>14.6 ± 2.2</td>
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</tr>
<tr>
<td>3T</td>
<td>8</td>
<td>80 ± 11</td>
<td>354 ± 55</td>
<td>18.5 ± 2.2</td>
<td>2478 ± 133</td>
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</tr>
<tr>
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<td>19.6 ± 1.8</td>
<td>2689 ± 312</td>
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</table>

a Control/TNF pairs with P values of 0.05 or less are italicized.

b The doses shown represent the total daily doses of TNF injected in μg/kg body weight (see "Materials and Methods" for further details).

c CAT activity is in kilounits.

d All rats in Experiments 4 and 5 were fasted on day 5.
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content when TNF is administered to rats at the concentrations

Kidney PER and CAT activities (in units/g protein) did not change significantly upon TNF administration in any of the five experiments (Table 3), while kidney G6PD activity showed relatively small increases (5 to 27% as compared to 31 to 80% in the liver) which, however, were statistically significant only in Experiments 2 and 3 (P < 0.05). LD activity showed decreases similar to those seen in the liver (2 to 17%) upon TNF administration with the largest decrease seen in the fasted rats (Experiments 4 and 5).

The ratio of catalatic to peroxidatic activity (or CAT/PER ratio) in the liver was relatively unchanged upon TNF administration (see Table 2), suggesting that the decrease in liver catalase did not involve a net dissociation or reassociation of the tetramer. It should be noted, however, that the liver CAT/PER ratio was on the average about 30% greater than that of the kidney (compare Tables 2 and 3), indicating that the liver may possess proportionately more catalatic activity, and correspondingly less peroxidatic activity, than the kidney.

Enzyme activities per g of protein correlated well with the enzyme activities per g of wet tissue and were on the average about 7-fold greater. As with the activities per g of protein, CAT and PER activities per g of wet tissue decreased significantly in the liver when the higher dose of TNF was administered (100 µg/kg/day) but did not change significantly in the kidney. Similarly there was a significant increase in G6PD activity in the liver and, to a much lesser extent, in the kidney. LD showed decreased activity in the liver and kidney which was more pronounced in the fasted rats. Finally, the ratio of catalatic to peroxidatic activity per g of tissue remained relatively unchanged upon TNF administration in both liver and kidney, and the ratio in kidney was about 30% higher than that in the liver. This similarity in results obtained by the two methods of calculation suggests that there was little change in tissue water content when TNF is administered to rats at the concentrations used in this study.

These results were confirmed in Experiment 6, where both pair-fed rats and rats fed ad libitum were used as controls to determine whether the decrease in food intake known to be induced by TNF may have an effect on the activity of CAT and PER in the liver. Blood was also collected from all the rats soon after sacrifice and the serum was separated and analyzed for CAT and PER activity to determine whether the decrease in the activity of these enzymes in the liver is reflected by increases in serum activity. As described under “Materials and Methods,” Experiment 6 included six rats given injections of the higher dose of TNF (50 µg/kg/day twice for 5 days), six pair-fed control rats, and four control rats fed ad libitum.

Daily mean body weights of the TNF-treated rats and pair-
fed rats were not statistically different from those of the rats fed ad libitum. As shown in Fig. 1, mean daily food consumption was also not significantly different between the TNF-injected rats (or pair-fed rats by definition) and the rats fed ad

\[ \text{CAT (units/liter) = 4.4 ± 1.1, PER (units/liter) = 8.0 ± 2.3} \]

\[ \text{(P ≤ 0.005)} \]

\[ \text{except on day 1, when the former consumed 5 g less food (P = 0.056). This decrease in food consumption represented a 20\% decrease in the mean daily food consumption of the rats fed ad libitum.} \]

Mean liver CAT and PER activities of the rats treated with TNF were 18 and 21\% lower than those of the pair-fed control rats (P = 0.01 and 0.001, respectively) and 23 and 21\% lower than those of the control rats fed ad libitum (P = 0.004 and 0.009, respectively). There was no statistically significant difference between the means of the pair-fed rats and the rats fed ad libitum.

Table 4 shows the corresponding activities in serum after correction for hemolysis as described in “Materials and Methods.” Mean serum CAT and PER activities in rats treated with TNF were 91 and 50\% greater, respectively, than those of the rats fed ad libitum (P = 0.01 and 0.02, respectively), and 42 and 33\% greater than those of the pair-fed rats (P = 0.09 and 0.04, respectively). It should be noted that the differences obtained for PER activity were probably more dependable than those obtained for CAT activity because the correction for contamination of serum by lyzed RBC represented a small fraction of the PER activity but a large fraction of the CAT activity (about 20 and 70\%, respectively; results not shown). This occurred because the CAT/PER ratio in the serum was very low (5- to 6-fold lower than that observed in the liver; compare Tables 4 and 2), suggesting that the subunits of the enzyme in the serum are present mainly in the dissociated form.

Ultrastructural studies were performed in Experiment 3 on the livers of the control rats and of the experimental rats given injections of 100 µg/kg/day TNF. Fig. 2 shows electron micrographs representative of the control and experimental livers, respectively. The control hepatocytes contained numerous per-
TUMOR NECROSIS FACTOR AND CATALASE

Fig. 2. A, electron micrograph of a hepatocyte from a control rat showing numerous large peroxisomes (arrows) and abundant cytoplasmic glycogen. Uranyl acetate, lead citrate, × 10,500; bar, 1 μm. B, hepatocyte from a TNF-treated rat showing only rare, small peroxisomes and scattered glycogen clusters. Uranyl acetate, lead citrate, × 10,500; bar, 1 μm.

Ultrastructural studies also showed that the loss in liver CAT and PER activity was accompanied by a decrease in the number and size of the peroxisomes in the hepatocytes where most of the catalase resides. There was also a reduction in the glycogen stores but no evidence of hepatocellular damage (Fig. 2). A TNF-induced decrease in glycogen content of the muscle cell line L6 has been shown by Lee et al. (20).

The decrease in liver CAT activity was not significantly altered by a 24-h period of starvation. This is consistent with the observations by Rechcigl and Price (21), who showed that liver catalase in the rat is unchanged after a 24-h period of starvation, but subsequently decreases gradually to a level of 50% after 7 days.

The CAT and PER activities of the kidney were relatively unchanged. Greenstein (7) and others reported that the decrease in the catalase activity of the liver of mice and rats with transplanted tumors is approximately 10 times greater than the decrease in the kidney. Since in our experiments there was a decrease of 21 to 56% in liver catalase activity (Table 2), the expected decrease in kidney catalase activity would be only 2 to 6%. Examination of the mean kidney CAT and PER activities in Table 3 indicates that statistically insignificant decreases occurred in four of the five experiments for CAT and three of the five experiments for PER.

Another significant change induced by TNF was an increase in the G6PD activity of the liver and, to a lesser extent, the kidney. The activity of this enzyme is regulated by the NADPH/NADP⁺ ratio and is completely inhibited at ratios greater than 9 (22). Since G6PD activity is usually assayed in the presence of the catalase resides. There was also a reduction in the glycogen stores but no evidence of hepatocellular damage (Fig. 2). A TNF-induced decrease in glycogen content of the muscle cell line L6 has been shown by Lee et al. (20).

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of a large excess of the substrate, NADPH, the velocity of the reaction is maximal. Therefore, the increase in enzyme activity may represent an increase in enzyme synthesis induced by oxidative stress, such as might be expected following treatment with TNF.

These differences in enzyme levels were essentially similar whether the activities were calculated per g of supernatant protein or per g of tissue wet weight, or whether pair-fed rats or rats fed ad libitum were used as controls. This is emphasized because TNF has been reported to induce fluid retention (5, 23). It should be noted, however, that in these studies the dose of TNF was either much greater than that used in the present study, resulting in histologically demonstrable liver damage, or the route of injection was i.v., resulting in higher concentrations of circulating TNF. In a recent report by Darling et al. (23), i.v. injection of 50 μg/kg/day TNF twice daily for 8 days (as compared to i.p. injection of the same daily dose for 5 days in the present study) caused a modest increase of only 8% in carcass water content.

Our results did not show a significant difference between the mean body weight of TNF-treated/pair-fed rats and that of control rats fed ad libitum, in contrast to the results of other investigators (24-26) who observed a transient decrease in body weight starting on day 1 after TNF injection. Daily food consumption of the TNF-treated/pair-fed rats was 20% lower than that of the control rats fed ad libitum on day 1 following TNF treatment (Fig. 1). This transient decrease, however, was only of borderline significance (P = 0.056; Fig. 1), but was similar to the decrease in food consumption reported by other investigators (24-26).

The reasons for the decrease in the CAT and PER activity of liver catalase following TNF administration remain speculative at this point. As indicated in the “Introduction,” TNF induces changes similar to those seen in cachexia and inflammation, where WBC undergo a respiratory burst with the generation of free radicals as a defense against a variety of invasive agents (27). The free radical scavenging enzymes such as catalase neutralize the oxidative effects of free radicals, but tissue damage occurs when the inflammatory response is large enough to exceed their capabilities (28). This was recently suggested by Oda et al. (29) who showed that the injection of superoxide dismutase conjugated to a pyran copolymer into mice protected against a potentially lethal influenza virus infection.

It may be postulated that TNF elicits inflammatory reactions that result in the movement of catalase from organs/tissues where it abounds, such as the liver and RBC, to areas of inflammation where free radicals are formed. This is consistent with our observation that the activity of CAT and PER in the serum of TNF-treated rats is significantly higher than that of pair-fed rats or control rats fed ad libitum. It is also consistent with the further observation that the CAT/PER in serum is about 5-fold lower than that in the liver, suggesting that the subunits of liver catalase are released into the circulation mainly in the dissociated form. There is other circumstantial evidence consistent with this hypothesis. First, Wohlaeb and Godin (30) have shown that oxidative stress induced in rats by a 72-h period of starvation resulted in a decrease in liver catalase activity and an increase in the catalase activity of the heart and pancreas. Although the increase in the latter two organs may be the result of de novo synthesis, it is conceivable that it is the result of movement of catalase from organs where it abounds, such as the liver, to areas where free radicals are formed. Second, it has long been known that serum catalase activity increases markedly in acute pancreatitis, even though the pancreas contains very little catalase (31). Here again the increase in serum catalase activity probably occurs while the enzyme is being transported to the site of inflammation. Third, the movement of catalase from one organ to another may also explain the recent observation by Rosenberg et al. (32) that erythrocyte catalase activity is diminished during rejection of transplanted kidneys. Fourth, Crane et al. (33) observed that enzymatically active catalase is derived from a larger molecule by the proteolytic cleavage of a polypeptide which he suggested may play a role in catalase transport across the cell membrane and thus facilitate its mobility.

The existence of a mechanism for the movement of catalase across cell membranes would explain the reason for the decrease in liver catalase activity in a variety of conditions that are seemingly unrelated. Close examination of such conditions, however, shows that they all have one factor in common, namely, the production of free radicals. In some conditions, free radical formation may be mediated through the action of cytokines such as TNF, as in the case of endotoxin shock, the presence of a tumor, and infection. In others, free radicals may be caused by the action of oxygen on fatty acids, as in severe starvation.

Further investigations are necessary to explore these possibilities, as well as other possibilities which might explain the decrease in catalase activity, including a decrease in the production of catalase by the liver (as by transcriptional, translational, or posttranslational control), or by a decrease in its activation.

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REFERENCES

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