Effects of interleukin 1-β (IL-1) and combination of IL-1 and tumor necrosis factor on tumor growth and protein metabolism

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This study examined the effects of the 20 μg/kg of interleukin-1 (IL-1), a combination of 10 μg/kg of IL-1 and 10 μg/kg of tumor necrosis factor (TNF), or 20 μg/kg of TNF on tumor growth and of IL-1 and the coinfusion on protein metabolism in the Yoshida sarcoma bearing rat. The results showed that IL-1 alone did not affect tumor growth. A significantly higher rate of tumor regression was seen in IL-1 and TNF coinfusion treated groups, as demonstrated by the fractional growth rate of −0.24 ± 0.05/day (P < 0.05). Twenty μg/kg of TNF alone had similar effects as coinfusion of the cytokines on tumor growth, indicated by the growth rate of −0.26 ± 0.03/day. In a second experiment, we measured the effects of IL-1 and IL-1/TNF combination on protein metabolism in both tumor and non-tumor tissues since the effect of TNF alone had been previously studied.[7] The data revealed that the observed antitumor effect of the coinfusion of cytokines was through decreases of tumor protein synthesis in both the periphery and center of the tumor tissue. In addition, IL-1 alone and the coinfusion reduced the fractional protein synthetic rate, and the rates of protein synthesis and breakdown in liver in both tumor bearing and healthy control rats, but did not cause liver protein loss. No effect was seen on muscle protein metabolism.

Keywords: interleukin 1; tumor necrosis factor; tumor growth; protein synthesis; protein breakdown

Introduction

Interleukin-1 (IL-1) and tumor necrosis factor (TNF) are cytokines secreted by the activated macrophage.[1,2] These cytokines share many biological activities such as fever production, redistribution of trace elements, endocrine alteration, and increased synthesis of acute phase proteins from liver.[3] Our previous studies have shown that administration of either 20 μg/kg of IL-1 and TNF separately, or the co-administration of the equivalent total dose, induced a similar pyrogenic response in rats.[4-6] In a recent study, we have demonstrated that administration of 20 μg/kg of TNF significantly increased tumor protein breakdown and inhibited tumor growth in Yoshida sarcoma bearing rats.[7] In order to compare the effects of cytokines on tumor growth and protein metabolism, the present study examined the effect of 20 μg/kg of human recombinant IL-1 alone, or a combination of 10 μg/kg IL-1 and 10 μg/kg TNF in Yoshida sarcoma bearing rats. Separately, 20 μg/kg of TNF alone was infused to determine its effect on tumor growth. Since the effect of this level of TNF on protein kinetics was measured previously in a similar tumor bearing rat model,[7] the effect of TNF on protein metabolism was not examined in the current study. In this study, protein metabolism was evaluated in tumor and host tissues by continuous infusion of 14-C-leucine, as previously described.[4-7] Tumor growth was estimated using daily measurements of tumor volumes.

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Materials and methods

Interleukin-1 (IL-1) and tumor necrosis factor (TNF)

Recombinant human interleukin-1 beta (IL-1) was kindly provided by Cistron Inc. (Pine Brook, NJ, USA) and recombinant human tumor necrosis factor-alpha (TNF) was generously provided by Genentech Inc. (San Francisco, CA, USA) (Lot No. 3055-56). The identity of these purified cytokines was confirmed by their amino acid composition and the sequence of the amino terminal heptapeptide by both suppliers.

Animals

All experiments were conducted in Sprague-Dawley weanling male rats, body weight 90–100 g (Taconic Farms Germantown, NY, USA). The tumor bearing animal model was created by the subcutaneous inoculation of 10^7 Yoshida sarcoma cells on day 0. A total of 60 rats were implanted with the tumor. Twenty-four control rats received subcutaneous sterile saline inoculation. Following these procedures, all animals were maintained on a standard commercial rat chow (Charles River D-3000; Agway Agricultural Products, Minneapolis, MN, USA) ad libitum and housed in a light and temperature controlled room for nine days. On day 9, all animals underwent surgical placement of a silastic catheter into the right jugular vein under light ether anesthesia. After surgery, these rats were returned to their cages and were fed ad libitum on rat chow. The patency of the catheter was maintained by a constant slow infusion of physiologic saline.

Experimental designs

The study consisted of two experiments. The purpose of the first experiment was to evaluate the rate of tumor growth after different treatments of cytokines during the 10th to 12th day after implantation. Forty tumor bearing rats were randomly divided into four groups. Each rat received a cytokine treatment on days 10 and 11. IL-1 and TNF solution were prepared freshly in a 0.1% human albumin saline solution to prevent its adherence to syringes and catheters. The control rats received 0.1% human albumin saline solution ("saline" in subsequent text).

Group I-1, control, received a 100 μl bolus injection of saline, followed by a four-hour constant infusion of saline at a rate of 1.2 ml/hr with a syringe pump (Harvard Apparatus Co., South Natick, MA, USA). Group I-2 received a bolus injection of 10 μg/kg of IL-1, followed by a constant infusion of IL-1 10 μg/kg for four hours. The total IL-1 dose was 20 μg/kg. Group I-3 received a 1:1 mixture of IL-1 (10 μg/kg) and TNF (10 μg/kg). Half of this mixture was injected as a bolus and the rest was constantly infused over four hours. Group I-4 received TNF 20 μg/kg given in the similar manner as Groups I-2 and I-3.

During the experimental period, all rats were housed in individual cages with free access to chow and tap water in a light and temperature controlled animal room. Following the daily cytokine infusion, physiologic saline was infused slowly for maintaining patency of the catheters. Tumor size was determined from day 10 to 12 by measuring its length, width, and depth using a caliper. On the 12th day, the rats were sacrificed and the tumor tissues were removed and weighed.

In the second experiment, the effects of IL-1 alone and a coinfusion of IL-1 and TNF on protein metabolism were determined in both tumor and non-tumor animals. Protein turnover studies were conducted on day 10 after implantation of tumor or saline. A total of 44 rats were divided randomly into 6 groups for different treatments:

Group II-1a (tumor rats) and Group II-1b (nontumor rats) received an injection of 100 μl of saline, followed by a four hour constant infusion of 1-^{14}C-leucine solution (1 uci/ml) at a rate of 1.2 ml/hr by a syringe pump.

Group II-2a (tumor rats) and Group II-2b (nontumor rats) received a bolus injection of 10 μg/kg of IL-1, followed by a constant infusion of 10 μg/kg of IL-1, and 1-^{14}C-leucine tracer as in Groups II-1a and II-1b.

Group II-3a and Group II-3b corresponded to Groups II-2a and II-2b, respectively, except that the cytokine consisted of a 1:1 mixture of IL-1 (10 μg/kg) and TNF (10 μg/kg).

All the animals were fasted overnight prior to the protein turnover study. During the isotope infusion, [$^{14}CO_2$] and total CO_2 production, VCO_2 were measured by methods described previously.[8] At the end of infusion, the animals were sacrificed by decapitation, and blood was collected into heparinized tubes and centrifuged. Plasma samples were stored at −20° C for further analysis. The animal body was dissected immediately after decapitation. The liver, and part of the abdominus rectus muscle, were removed and weighed. Each liver and muscle sample was divided into two fractions; one piece was placed in 5 ml of 10% sulfosalicylic acid, and another in 5 ml of physiologic saline. These tissue samples were frozen immediately in liquid nitrogen and stored at −20° C until analysis. Similarly, the whole tumor was removed, weighed, and divided into central and peripheral parts, and then preserved in liquid nitrogen.

Assays

Plasma samples were analyzed for free leucine specific activity according to the methods described before.[9] Briefly, plasma proteins were precipitated with 30% sulfosalicylic acid (SSA) and subsequently centrifuged. Leucine concentration (μmol/ml) in plasma was analyzed by HPLC using precolumn orthophthaldialdehyde derivatization and fluorescence detection by microbondapak C18 column. The radioactivity of leucine in the supernatant was determined by Beckman LS-8000 liquid scintillation counter (Beckman Instruments, Fullerton, CA, USA) after removal of alpha-ketoisocaproic acid with 30% hydrogen peroxide.

Tissue stored in 10% SSA was homogenized and centrifuged. The specific activities of leucine in the acid-soluble tissue fraction (Si) were determined using a similar method as described above for plasma samples. Protein bound specific activities (Sb) in these tissues were determined by drying the precipitates and redissolving them into tissue solubilizer (Beckman BTS-450, Beckman Instruments, Fullerton, CA, USA). The solutions were then counted and the leucine concentrations determined as described previously.[9]

Tissues stored in saline were used to determine total nitrogen content using the micro-Kjeldahl method.[9]

Calculations

Tumor growth rate. Changes in tumor volume (V) were expressed as the product of tumor dimension:

$$V = L \times W \times H \quad (1)$$

where L is the length of tumor, W is the width of tumor, and the H is the depth of the tumor.

The fractional rate of tumor growth, kg, was then estimated from the change in tumor volume between days 10 to 12 after implantation, according to the following equation:

$$Vt = Voe^{kgt} \quad (2)$$

where Vt is the tumor volume on day 12, Vo is the tumor volume on day 10, and t is the period of 2 days.

Plasma leucine kinetics. Plasma leucine appearance was calculated from the dilution of radioactivity leucine in the plasma compartment using the equation for whole body leucine kinetics.[8,10]

$$Q = I/Sp\ max \quad (3)$$

where Q is the flux of leucine appearance and disappearance in the plasma compartment, I is the infusion rate of L-[1-^{14}C]-leucine, and $Sp\ max$ is the plasma leucine specific activity at steady state.

Based on the circulation leucine flux (Q), whole body protein metabolism can be divided further into its components according to the following equation:

$$Q = I + B = S + O \quad (4)$$

where I is dietary leucine, B is the contribution from body protein breakdown, S is the rate of utilization of plasma leucine in protein synthesis, and O is its oxidation rate. In this study, I equals zero since the study was conducted on post-absorptive stage. The oxidation of leucine derived from the plasma compartment was estimated from the appearance of $^{14}CO_2$ in the expired breath using the following equation:

$$O = (E/Sp\ max)/0.81 \quad (5)$$

where O is the oxidation rate, E is the appearance rate of $^{14}CO_2$, and 0.81 is the percentage of the metabolically generated carbonate that appears in expired breath.[8,10]

Tissue leucine kinetics. The rates of protein synthesis in liver, muscle, and tumor were calculated from the following equation[11]:

$$\frac{S_b}{S_i} = \frac{\lambda_i}{(\lambda_i - K_s)} \cdot \frac{(1 - e^{-K_s t})}{(1 - e^{-\lambda_i t})} - \frac{K_s}{(\lambda_i - K_s)} \quad (6)$$

where K_s is the fractional rate of protein synthesis (FSR), λ_i is the rate constant for the increase in radioactivity in the intracellular-free amino acid pool, and t is the duration of tracer infusion.

Statistics

Results are summarized as mean ± SEM. Statistical analysis was performed using the BMDP statistical software package (Regents of University of California, Los Angeles, CA, USA, 1983). In experiment one, tumor growth rates were compared by one-way analysis of variance. In experiment two, the differences of protein kinetics among normal and tumor bearing rats receiving different treatments were compared by two-way analysis of variance, and inter-group comparisons were made by Student t test when there was overall significance with the two-way ANOVA.

Results

In the first experiment, changes in tumor growth, between days 7–12 after implantation are presented in *Figure 1* for the different experimental groups. From days 7 –10, a constant expansion of tumor size was observed, indicating the progressive growth of Yoshida sarcoma in rats. However, tumor volume was reduced slowly from 4.18 ± 0.57 cm^3 to 3.87 ± 0.49

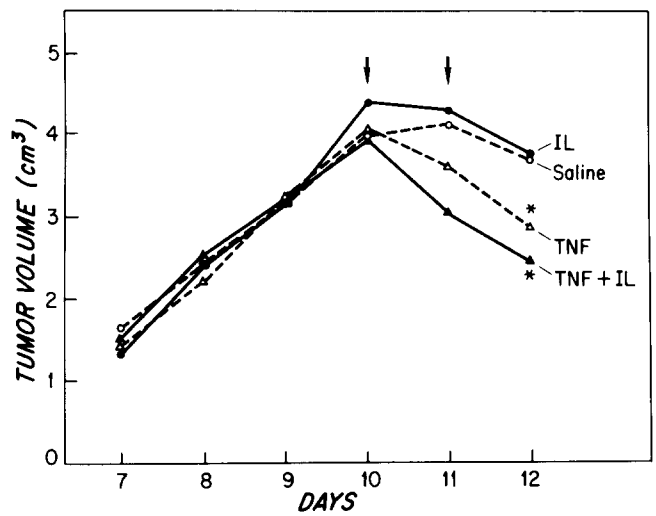

Figure 1 Tumor growth of Yoshida sarcoma in rats before and after treatments on day 10 and day 11 with saline (○ – – ○), 20 μg/kg of IL-1-alone (●—●), a mixture of 10 μg/kg of IL-1 and 10 μg/kg of TNF (▲—▲), or 20 μg/kg of TNF alone (△ – – △). Points are average values from 10 rats. Data were presented as mean ± SE; the range of SE from 0.24 to 0.57 * denotes significant difference compared with the saline and IL-1 treatments ($P < 0.05$)

Table 1 Estimates of leucine kinetics in tumor tissue

Group	Fractional Synthetic Rate (%/day)		Si/Sp	
	Periphery	Center	Periphery	Center
II-1a (6) (saline)	70.90 ± 6.71	33.75 ± 7.36	0.24 ± 0.09	0.13 ± 0.05
II-2a (6) (IL-1)	76.67 ± 7.10	39.12 ± 8.42	0.20 ± 0.03	0.09 ± 0.06
II-3a (8) (IL-1 + TNF)	40.13 ± 5.23*	12.16 ± 2.08†	0.27 ± 0.03	0.21 ± 0.14

Note. Mean ± SE.
* $P < 0.005$, vs. II-1a and II-2a by one-way ANOVA.
† $P < 0.05$, vs. II-1a and II-2a by one-way ANOVA.

Table 2 Estimates of whole body leucine kinetics in Yoshida-sarcoma-bearing rats

Group	Flux (μmol/hr)	Synthesis (μmol/hr)	Oxidation (μmol/hr)	% of Flux Oxidized
II-1a (6)	72.65 ± 9.58	49.43 ± 5.42	23.29 ± 4.63	30.70 ± 2.21
II-1b (8) (saline)	67.78 ± 5.48	46.91 ± 2.62	20.87 ± 3.01	30.22 ± 1.89
II-2a (6)	76.78 ± 5.87	54.01 ± 3.08	21.96 ± 3.08	27.80 ± 1.90
II-2b (8) (IL-1)	75.70 ± 3.97	49.54 ± 2.53	26.17 ± 1.37	34.62 ± 1.00
II-3a (8)	60.51 ± 7.61	46.97 ± 2.36	23.22 ± 1.23	33.26 ± 1.11
II-3b (8) (IL-1 + TNF)	70.19 ± 3.94	40.03 ± 5.17	20.47 ± 2.83	33.74 ± 1.89

Note. Mean ± SE.
a: Non-tumor rats.
b: Tumor-bearing rats.

cm^3 in the saline-treated group (Group I-1) after day 10. The mean fractional growth rate was -0.17 ± 0.16/day. During the same period of time, Group I-2, which received two consecutive intravenous infusions of IL-1 (on day 10 and day 11), showed a similar fractional growth rate of -0.15 ± 0.09/day, which was not significantly different from the saline treated group. The group that received a mixture of IL-1 and TNF (Group I-3) showed a significantly increased rate of tumor regression, as demonstrated by the fractional growth rates of -0.26 ± 0.03/day ($P < 0.001$ versus saline and IL-1 by one-way ANOVA). Comparison of the combination with TNF alone revealed no significant difference in tumor growth, since the growth rate of tumor was -0.24 ± 0.05/day.

From experiment two, estimates of protein kinetics in tumor tissues are presented in *Table 1*. In saline treated group (Group II-1a), the fractional protein synthetic rate in the peripheral tumor portion was twice as high as that in the central necrotic part. Additionally, the ratio Si/Sp of free radioactive leucine was significantly lower in the central part of the tumor. In a tissue synthesizing protein at a lower rate, such as in the tumor center, the lower Si/Sp could only be contributed by dilution from protein breakdown.[5,12,13] Therefore, our results indicate significant regional differences in protein synthesis and breakdown in the tumor tissue with the central part having a lower rate of protein synthesis and a higher rate of protein breakdown than the peripheral part.

Comparison of Group II-1a and II-2a shows that IL-1 did not affect protein synthesis or protein breakdown in the tumor tissue, either in the peripheral part or in the central part. In contrast, as shown in *Table 1*, when the tumor bearing animals received a mixture of TNF and IL-1, a significant decrease in the fractional protein synthetic rate was observed in both tumor periphery and center. However, there were no significant changes in the ratio of Si/Sp in these two parts in this group. These results show that the effects of a mixture of IL-1 and TNF on tumor growth in Yoshida-sarcoma-bearing rats are reflected in tumor protein synthesis.

At the level of circulating plasma, whole body protein kinetics did not significantly differ among the groups (*Table 2*). Protein kinetics of individual host tissues, liver, and muscle, are shown in *Tables 3* and *4*. The liver weight was significantly higher in the tumor bearing groups (Groups II-1a, II-2a, and II-3a) than non-tumor groups (Groups II-1b, II-2b, and II-3b) ($P < 0.05$ by two-way ANOVA) (*Table 4*). However, the fractional protein synthesis rates, as well as total liver protein content, were similar between tumor bearing and control animals (*Table 3*). Infusions of either 20

Table 3 Estimates of leucine kinetics in liver and muscle in normal weanling rats and in Yoshida-sarcoma-bearing rats

Group	Liver		Muscle	
	FSR(%/day)	Si/Sp	FSR(%/day)	Si/Sp
Il-1a (6)	56.29 ± 6.55	0.36 ± 0.04	6.41 ± 0.67	0.18 ± 0.02
Il-1b (8) (saline)	54.88 ± 6.05	0.36 ± 0.04	7.41 ± 1.05	0.22 ± 0.03
Il-2a (6)	35.93 ± 2.37*	0.42 ± 0.04*	5.67 ± 0.84	0.13 ± 0.09
Il-2b (8) (IL-1)	32.32 ± 6.73*	0.61 ± 0.05*	6.21 ± 0.91	0.16 ± 0.02
Il-3a (8)	38.08 ± 4.95*	0.59 ± 0.08*	6.56 ± 1.61	0.23 ± 0.04
Il-3b (8) (IL-1 + TNF)	35.01 ± 1.18*	0.51 ± 0.78	5.82 ± 0.75	0.22 ± 0.02

Note. Mean ± SE.
a: Non-tumor rats.
b: Tumor-bearing rats.
* $P < 0.05$, vs. saline by two-way ANOVA.
FSR: Fractional synthetic rate; Si/Sp: Ratio of leucine specific activity in acid soluble tissue fraction to plasma specific activity at isotopic steady state.

Table 4 Protein composition and estimates of protein synthesis rates in liver in normal weanling rats and in Yoshida-sarcoma-bearing rats

Group	Weight g	Total Protein g	Synthesis μmol/hr
Il-1a (6)	5.12 ± 0.15	1.10 ± 0.06	18.30 ± 2.81
Il-1b (8) (saline)	4.58 ± 0.14*	1.09 ± 0.03	17.51 ± 1.98
Il-2a (6)	5.47 ± 0.23	1.15 ± 0.05	12.27 ± 1.19†
Il-2b (8) (IL-1)	4.71 ± 0.17*	1.13 ± 0.04	10.63 ± 2.28†
Il-3a (8)	5.30 ± 0.14	1.20 ± 0.04	11.95 ± 2.01†
Il-3b (8) (IL-1 + TNF)	5.08 ± 0.16*	1.09 ± 0.06	11.55 ± 0.78†

Note. Mean ± SE; a: Non-tumor rats; b: tumor-bearing rats.
* $P < 0.001$, vs. non-tumor by two-way ANOVA;
† $P < 0.005$, vs. saline by two-way ANOVA.

μg/kg of IL-1 alone, or a mixture of IL-1 and TNF, significantly reduced the fractional protein synthetic rate and the rate of total protein synthesis in the liver, and increased the ratio of Si/Sp in this tissue as compared to the saline infusion, regardless of whether animals were tumor bearing or not. Since the protein content in the liver was not affected by infusion of monokines (*Table 4*), these results suggest that IL-1 alone or a mixture of IL-1 and TNF may diminish the rate of protein synthesis and breakdown in liver in tumor bearing rats, as well as in non-tumor bearing rats, so that no net catabolic losses were observed following infusion of cytokines. In the muscle, neither IL-1 alone nor a 20 μg/kg mixture of IL-1 and TNF affected protein kinetics in tumor bearing or non-tumor bearing animals, as compared to saline treatment groups.

Discussion

The results reported here indicate that administration of recombinant IL-1 alone to Yoshida-sarcoma-bearing rats had no significant effect on tumor regression as compared to saline control. In this study, IL-1 was infused at a dose level of 20 μg/kg, by an initial bolus injection of 10 μg/kg of IL-1, followed by a constant infusion of an additional 10 μg/kg of IL-1 for four hours. This dose of systemically infused IL-1 did not affect tumor protein synthesis or breakdown as compared to saline infusion. It appears that the Yoshida sarcoma tumor does not respond to early exposure to IL-1 in the rat. Furthermore, since two consecutive infusions of IL-1 at an interval of 24 hr did not change the growth rate of Yoshida sarcoma between days 10–12 after implantation, it suggests that this tumor is also insensitive to IL-1 after repeated exposure in the rat. In contrast to IL-1 alone, substitution of 50% of IL-1 by TNF or 100% replacement of IL-1 by TNF on two consecutive days significantly increased the regression of Yoshida sarcoma in rats. There was a similar depression of tumor growth by these two different treatments. It is also noted that the tumor regression induced by 50% of IL-1 or TNF of each was associated with measurable decreases in tumor protein synthesis (*Table 2*), while the results from our previous study[7] showed that the regression of tumor induced by 100% TNF was associated with the increases in tumor protein breakdown.

The reason why IL-1 alone has no antitumor effects on Yoshida sarcoma tumor tissue in rats is unclear. One possibility may be related to the different receptor bindings of these two cytokines on the tumor cells. Many biologically active molecules, including hormones and lymphokines, need first to bind to specific cell surface receptors[14,15] to ensure their effects on these cells. TNF and IL-1 have independent receptors on various cells.[16] Therefore, it is possible that the Yoshida sarcoma cell lacks a receptor for IL-1 but has one for TNF. An alternative explanation is that the circulating and local IL-1 may not have reached a sufficient level to enhance the cellular signal subsequent to receptor binding. A dose-dependent cytotoxic effect of IL-1 on human breast tumor cell line has been

reported by Gaffney and Tsai[17,18] in vitro at concentrations greater than 4 U/ml. Onazaki et al.[19] and Lanchman et al.[20] have also found that IL-1 inhibits growth of a human melanoma cell line only at concentration equal or greater than 2 U/ml. However, these possibilities will not be validated until IL-1 receptors are further characterized on Yoshida sarcoma tumor cells.

In view of evidence for stimulation of IL-1 secretion by TNF administration,[21-23] the combined effects of these structurally distinct cytokines are often greater than either one alone. Thus, an amplification of antitumor effect might be contributed by the co-existence of TNF and IL-1 in vivo. This hypothesis is in line with our observation that combined IL-1 and TNF administration had potential antitumor effects as compared to IL-1 alone, since substitution of IL-1 with 50% of TNF showed a significant effect on tumor protein metabolism and on tumor growth (*Table 2* and *Figure 1*). However, it remains unclear whether the response of tumor growth after 50% replacement IL-1 was due to the effects of both cytokines or solely to the remaining half-dose of TNF. Further studies on dose response in tumor growth and protein metabolism to TNF alone would be one approach to answering the questions.

The results of the present study also showed that administration of IL-1 alone into healthy rats did not affect the skeletal muscle protein synthesis and breakdown (Group II-2b). This observation is in agreement with those reported by other investigators. Several studies from our laboratory have demonstrated that administration of 20 μg/kg of IL-1 alone did not increase either amino acid release from protein degradation or oxidation.[4-6] We have hypothesized that purified preparations of biologically derived IL-1 used in the past may have been contaminated with other cytokines, particularly TNF, so that recombinant IL-1 failed to demonstrate catabolic effects on protein as previously reported. Recently, Moldawer et al.[24] and others[25] have reported that purified recombinant IL-1 did not increase release of tyrosine from muscle in vitro and in vivo. Some product of activated blood monocytes, immunologically distinct from IL-1, may be responsible for the accelerated protein degradation that occurs in vitro, and in vivo. Wilmore and colleagues[26] also reported that etiocholanolone administration to healthy volunteers resulted in fever and increased plasma levels of interleukin-1 without increase in the net release of amino acids from skeletal muscle. Our data further support these observations showing that IL-1 did not have a catabolic effect on muscle protein metabolism in tumor bearing animals.

This study demonstrated that the effects of a coinfusion of IL-1 and TNF is tissue-specific, since protein synthesis and protein mass were decreased significantly in tumor tissue (*Table 1*), but were not altered in muscle tissue (*Table 3*). The lack of response was also noted in muscle tissue of the non-tumor bearing rats (Group II-3b), a finding which is at variance with other published reports from our laboratory. While the exact reason of this discrepancy cannot be determined, it is important to note that the rats used in the current study were significantly younger than the rats of Flores et al.[5] As listed in *Table 3*, muscle fractional synthetic rates were 2–3 times larger than those reported by Flores et al., thus raising the possibility of a relationship between muscle protein turnover and the dose of TNF or coinfusion necessary to induce net muscle catabolism. Similarly, the lack of catabolic effect for TNF in muscle tissue reported by Goldberg et al.[25] could be related to the fact that these investigators also used young rats.

The lack of catabolic response in liver protein content in the current study also contrasts with our earlier findings,[5] and those of others (Fong, et al., *Am. J. Physiol.* **256,** R659–665). In addition to the possible reasons discussed above for muscle tissue, it is noted that the pool of liver protein analyzed in the present study is a mixture of secretory as well as non-secretory proteins. Further separation of these proteins is needed to better characterize the effect of cytokines on the liver.

In summary, the results from the present study suggest that the in vivo effects of TNF and IL-1 on tumor and host tissue protein metabolism are different and appear to involve a number of undefined variables.

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