The effects of recombinant tumour necrosis factor (cachectin) on metabolism in isolated rat adipocyte, hepatocyte and muscle preparations

Allan M. ROFE,* Robert A. J. CONYERS,*† Renze BAIS,* Jennifer R. GAMBLE† and Mathew A. VADAS†

*Metabolic Research Group, Division of Clinical Chemistry, and †Division of Human Immunology, Institute of Medical and Veterinary Science, Frome Road, Adelaide, S.A. 5000, Australia

Tumour necrosis factor (TNF) did not stimulate lipolysis in isolated rat adipocytes, though preincubation with TNF increased adrenaline-stimulated fatty acid release. Glycogenolysis, gluconeogenesis and ketogenesis in isolated rat hepatocytes were not influenced by TNF in short-term (30–60 min) incubations. TNF stimulated $^{14}\text{CO}_2$ production from $[\text{U-}^{14}\text{C}]\text{glucose}$ in rat hemidiaphragm preparations, but lactate production and alanine release were not significantly altered. It is concluded that TNF does not regulate short-term metabolism in adipocytes, hepatocytes and muscle preparations in the manner of a catabolic hormone.

INTRODUCTION

Neoplastic and infectious diseases may lead to host cachexia, which is characterized in particular by muscle and adipose-tissue wasting and which may be accelerated by anorexia, therapy or secondary infection. By comparison with simple starvation, the weight loss and anorexia are accompanied by accelerated muscle proteolysis and adipose-tissue lipolysis, and by increased hepatic rates of fatty acid oxidation and gluconeogenesis from lactate, amino acids and glycerol [1–5]. These catabolic effects are often inappropriately excessive for the tumour or microbial pathogen burden, and have led investigators to seek endogenous host metabolic [6–8] or immune [9] mechanisms.

In response to a number of invasive stimuli, a variety of cells, including macrophages and lymphocytes, secrete cytokines which are capable of altering host metabolism (see [9,10]). These cytokines include interleukin-1 (IL-1), γ -interferon, lymphotoxin (TNF- β) and tumour necrosis factor (TNF- α). Cachectin, a protein released from endotoxin-stimulated macrophages, has been shown to be homologous with TNF- α and has, among its other properties, the ability to inhibit the synthesis of lipoprotein lipase in transformed mouse fibroblast/adipocyte (3T3-L1) cells [9]. TNF- α /cachectin (TNF) is several orders of magnitude more potent than IL-1, γ -interferon or TNF- β in inhibiting the activity of lipoprotein lipase in 3T3-L1 cells and has now been shown to stimulate lipolysis directly in these cells [10]. Such findings have implicated TNF as a potential modulator of the adipose-tissue wasting in cancer and infection [9]. These studies of the lipid-mobilizing activity of TNF, although using purified human recombinant preparations ('rHuTNF- α '), have been restricted to the examination of the effects of TNF on the one transformed mouse (3T3-L1) cell line (see [9,10]). We present here an examination of the effects of recombinant human TNF- α on fatty acid, glucose and alanine metabolism in preparations *in vitro* of adipocytes,

hepatocytes and diaphragm muscles isolated from the rat. We have used these three normal tissues, which have major roles in the cachectic wasting process, to determine if TNF acts in a non-time-dependent manner analogous to catabolic hormones.

MATERIALS AND METHODS

The recombinant human TNF- α was generously provided by Genentech, San Francisco, CA, U.S.A. (lot no. 3056-55, containing 2.5×10^7 units/ml; 0.5 mg/ml). The sources of all other materials and rats, the animal housing conditions and the dietary composition have been described previously [7,8,11]. Adipocyte [12] and hemidiaphragm-muscle [13] studies were performed with tissue taken from male Porton rats (100–300 g) fed *ad libitum*. Hepatocytes were isolated from female Dark Agouti rats (150–200 g) [8], fed *ad libitum* or starved. Glycogenolysis was measured by assaying the rate of glucose release in the absence of substrate. The measurement of the metabolism of $[\text{U-}^{14}\text{C}]\text{palmitate}$ and the enzymic assays of metabolites in neutralized HClO_4 extracts of either cell suspensions or muscle incubation medium were performed as described previously [8,11]. Results are reported as means $\pm$ S.E.M. for the test, expressed as a percentage of the control values, with the numbers of separate preparations shown in parentheses. Statistical difference from the control value was assessed by the paired *t* test and checked with the Wilcoxon Signed Rank test. In only two cases (see below) was a discrepancy noted.

RESULTS

Recent studies have shown that concentrations of recombinant human TNF- α in the picomolar range have marked effects on metabolism in mouse 3T3-L1 cells, with maximal effects on lipoprotein lipase activity and

Abbreviations used: TNF, tumour necrosis factor (cachectin); IL-1, interleukin-1.

† To whom correspondence and reprint requests should be addressed.

acetate uptake occurring at 1 nM-TNF [10]. Blood concentrations of approx. 0.3 nM-TNF have been recorded in patients with parasitic infections and cancer [14]. From our preliminary screening experiments with a range of concentrations (0.01–2.4 nM) of the cytokine, we chose to use 1.2 nM-TNF in all subsequent experiments. Cell and muscle incubations were, with only one exception (see the adipocyte experiments), performed without preincubation in the presence of TNF and were all of short duration (30–120 min).

Adipose-tissue metabolism

The effects of TNF on lipolysis (glycerol release) and glucose oxidation ($^{14}\text{CO}_2$ production from $[\text{U-}^{14}\text{C}]$ -glucose) were examined in isolated adipocytes (Table 1). Viability of the rat adipocytes was not affected by the TNF treatment (Trypan Blue exclusion). TNF did not significantly affect metabolism under conditions where adrenaline caused a 10- and 4-fold stimulation of lipolysis and glucose oxidation, respectively, and insulin a 2-fold stimulation of glucose oxidation. The simultaneous addition of TNF did not affect these hormone-mediated effects (results not shown). However, it was noted that preincubation of adipocytes with TNF for 1 h enhanced the lipolytic effect of adrenaline (20% increase compared with no preincubation; not significant). In these experiments, the measurement of lipolysis by glycerol release was confirmed by the parallel measurement of fatty acid release (see [12] for method). This effect on fatty acid release (29% increase) was not significant with the paired *t* test, but it was with the Wilcoxon Signed Rank test ($P < 0.05$).

Liver metabolism

Isolated hepatocytes were incubated with TNF for 30 min, and the rates of glycogenolysis, gluconeogenesis, ketogenesis and the partition of palmitate between the pathways of oxidation and esterification were measured. Cell viability (Trypan Blue exclusion) was unchanged by the TNF treatment. In preliminary experiments with hepatocytes isolated from fed rats, adrenaline (50 μM) produced a 2-fold stimulation of glucose release. Under the same conditions, TNF had no effect (Table 2). TNF was essentially without effect on gluconeogenesis, ketogenesis and palmitate metabolism in hepatocytes from starved rats.

Table 1. Effect of TNF- α on rat adipocyte metabolism

The results are shown as percentages of the control in parallel incubations, and are means $\pm$ S.E.M., with the numbers of individual tissue preparations shown in parentheses. Concentrations used were TNF- α , 1.2 nM, adrenaline, 1 μM , and insulin, 6.4 nM. * $P < 0.05$, paired *t* test.

	Metabolism (% of control)	
	Glycerol release	$^{14}\text{CO}_2$ produced from $[\text{U-}^{14}\text{C}]$ -glucose
TNF- α	111 $\pm$ 6 (3)	100 $\pm$ 5 (3)
Adrenaline	1080 $\pm$ 109 (4)*	405 $\pm$ 38 (4)*
Insulin	—	219 $\pm$ 7 (3)*

Table 2. Effect of TNF- α on hepatocyte metabolism

The results are shown as percentages of the control in parallel incubations, and are means $\pm$ S.E.M., with the numbers of individual tissue isolations shown in parentheses. Glycogenolysis was measured in hepatocytes from fed rats. Hepatocytes from 24 h-starved rats were used in the other experiments. The concentrations used were lactate, 10 mM, palmitate, 1 mM, and TNF- α , 1.2 nM. Incubation time was 60 min for glycogenolysis and 30 min for all other experiments.

	Metabolism (% of control)
Glycogenolysis	97 $\pm$ 5 (4)
Gluconeogenesis	
From lactate	110 $\pm$ 9 (3)
From alanine	98, 98
From glycerol	103, 105
Ketogenesis from palmitate	105 $\pm$ 4 (3)
Conversion of $[\text{1-}^{14}\text{C}]$ palmitate into:	
$^{14}\text{CO}_2$	107 $\pm$ 3 (3)
^{14}C -labelled esterified products	99 $\pm$ 4 (3)
^{14}C -labelled ketone bodies	102 $\pm$ 4 (3)

Table 3. Effect of TNF- α on muscle metabolism

The results are shown as percentages of the control in parallel incubations, and are means $\pm$ S.E.M., with the numbers of individual tissue preparations shown in parentheses. The concentrations used were glucose, 10 mM, and TNF- α , 1.2 nM. * $P < 0.05$, paired *t* test.

	Metabolism (% of control)
$[\text{U-}^{14}\text{C}]$ Glucose $\rightarrow$ $^{14}\text{CO}_2$	131 $\pm$ 12 (6)*
Glucose $\rightarrow$ lactate	99 $\pm$ 5 (6)
Alanine release	111 $\pm$ 9 (6)

Muscle metabolism

The oxidation of $[\text{U-}^{14}\text{C}]$ glucose to $^{14}\text{CO}_2$ in rat hemidiaphragms was increased by 31% in the presence of TNF ($P < 0.05$ by paired *t* test; $P > 0.05$ by Wilcoxon Signed Rank test) (Table 3). Lactate production from glucose and alanine release to the medium were not significantly altered by TNF.

DISCUSSION

Cachexia is characterized by altered whole-body metabolism, with the liver, muscle and adipose tissue having central roles in the provision and interconversion of energy substrates [1–5]. If TNF- α /cachectin has a role in this wasting process, one might expect to observe effects in these tissues in a manner analogous to those changes elicited by catabolic hormones. Apart from a significant but small stimulation of CO_2 production from glucose in muscle, the general conclusion from this study is that TNF does not appear to behave in this manner.

Some caveats, however, could be applied to the interpretation of these results. Although it is possible that the enzymic treatment used to isolate the adipocytes and hepatocytes may have affected the receptors for TNF, the hepatocytes were still responsive to adrenaline and the adipocytes to both adrenaline and insulin, suggesting that such a non-specific effect is unlikely. The lability of TNF, which has a 6 min half-life *in vivo*, must also be considered. The stability of TNF under the incubation conditions used here is not known, but should not be different from that in studies of a similar type [10,15,16]. Since the amount of TNF used in the present studies was far in excess of that required to demonstrate significant metabolic effects in 3T3-L1 cells (major inhibition of acetate uptake at 0.001–0.01 nM-TNF) [10], it can be concluded that significant TNF activity would have been present in these experiments. The TNF used here has been shown by us to be functional in other assays, such as neutrophil adherence, superoxide release and chemotaxis (see [15,16]).

Most studies *in vitro* to date have examined metabolic changes after pre-exposure of 3T3-L1 cells to TNF for periods of at least 24 h. In one study [10], however, it was reported that TNF affected lipolysis in 3T3-L1 cells after only brief (1 h) preincubations with TNF. Although preincubation with TNF did not directly enhance lipolysis in rat adipocytes, it did increase the response of these cells to the lipolytic action of adrenaline. In the metabolic setting of a stressed catabolic disease state, this effect would facilitate the availability of fatty acid in the circulation to meet energy demands.

The possibility that cytokines may influence metabolism in the liver by indirect rather than direct mechanisms must also be considered. For example, the cytokine IL-1 has been reported to affect only alanine uptake in isolated rat hepatocytes, but, if the rats were first pre-treated with IL-1, hepatocytes isolated from those animals showed marked changes in energy expenditure and gluconeogenesis [17]. The investigators concluded that the effects observed may have been the result of IL-1 eliciting an endocrine response, which in turn influenced hepatic metabolism. Therefore the lack of a direct response to TNF in the hepatocyte experiments reported here does not negate the possibility that TNF may affect metabolism indirectly by modulating hormone effects *in vivo*.

It has been shown that TNF lowers plasma membrane potential in skeletal muscle [18]. Our finding of enhanced glucose oxidation to CO₂ in diaphragm muscle in the presence of TNF is consistent with increased mitochondrial oxidation resulting from increased ATP consumption for the maintenance of membrane potential. This redirection of energy flux could lead to decreased protein synthesis and increased protein catabolism. The observation of a small increase in alanine release from muscle in the presence of TNF is therefore worthy of closer study.

Although it is attractive to suggest that a single mediator may elicit a range of metabolic effects in the host [9], such hypotheses must also be tempered with evidence for the presence of significant amounts of such mediators in the cachectic diseases in question. In a recent study [16] it was found that only 8% of cancer patients had increased plasma TNF, a value identical with that observed in controls, whereas over 65% of patients with parasitic infections (visceral leishmaniasis,

malaria) had increased TNF. However, the degree of wasting in the cancer patients used in the study was not noted, although patients ranged from those in remission to ones with advanced disease. Other workers have not found increased TNF in cancer patients [19].

We conclude that TNF has no major effects on short-term metabolism in isolated adipose, liver and muscle preparations under incubation conditions in standard physiological buffers. It is possible that metabolic effects may be elicited by more complex incubation conditions or extended exposure to the cytokine. However, our aim was to examine potential effects of the peptide in commonly used metabolic models which have been the basis of much of our current understanding of metabolic regulation. TNF may still be important in modulating the wasting syndrome via effects on lipoprotein lipase activity and lipolysis, but such effects need to be shown to occur in metabolic systems other than 3T3-L1 cells. The conclusion from this study is that TNF probably does not act like a catabolic hormone, and its direct effects in this regard, if any, are probably minor in comparison with those of established hormones in determining cachexia. The negative findings reported here highlight the need for investigators to determine the specific properties of this highly purified and commercially available cytokine before it falls into disrepute clinically through studies based on the more global properties implied in its present names TNF/cachectin [20,21,22].

We gratefully acknowledge the support of the Anti-Cancer Foundation of the Universities of South Australia, and thank Catherine Bourgeois for her technical assistance.

REFERENCES

- Stein, T. P. (1978) *J. Theor. Biol.* **73**, 51–59
- Conyers, R. A. J., Need, A. G., Durbridge, T., Harvey, N. D. M., Potezny, N. & Rofo, A. M. (1979) *Med. J. Aust.* **1**, 398–399
- Magee, B. A., Potezny, N., Rofo, A. M. & Conyers, R. A. J. (1979) *Aust. J. Exp. Biol. Med. Sci.* **57**, 529–537
- Brennan, M. F. (1981) *N. Engl. J. Med.* **305**, 375–382
- Lawson, D. H., Richmond, A., Nixon, D. W. & Rudman, D. (1982) *Annu. Rev. Nutr.* **2**, 277–301
- Conyers, R. A. J., Potezny, N., Atkinson, E., Anargyros, P., Rofo, A. M. & Bais, R. (1981) in *Infection in Surgery* (Watts, J. McK., McDonald, P. J., O'Brien, P. E., Marshall, V. R. & Findlay-Jones, J. J., eds.), pp. 91–93, Churchill Livingstone, London
- Rofo, A. M., Porter, S. J., Bais, R. & Conyers, R. A. J. (1985) *Br. J. Cancer* **52**, 619–623
- Rofo, A. M., Bais, R. & Conyers, R. A. J. (1986) *Biochem. J.* **233**, 485–491
- Beutler, B. & Cerami, A. (1986) *Nature (London)* **320**, 584–588
- Patton, J. S., Shepard, H. M., Wilking, H., Lewis, G., Aggarwal, B. B., Eessalu, T. E., Gavin, L. A. & Grunfeld, C. (1986) *Proc. Natl. Acad. Sci. U.S.A.* **83**, 8313–8317
- Rofo, A. M. & Williamson, D. H. (1983) *Biochem. J.* **212**, 231–239
- Rofo, A. M. & Williamson, D. H. (1983) *Biochem. J.* **212**, 899–902
- Goldstein, L. & Newsholme, E. A. (1976) *Biochem. J.* **154**, 555–558
- Scuderi, P., Sterling, K. E., Lam, K. S., Finley, P. R., Ryan, K. J., Ray, C. G., Petersen, E., Slymen, D. J. & Salmon, S. E. (1986) *Lancet* **ii**, 1364–1365

15. Gamble, J. R., Harlan, J. M., Klebanoff, S. J. & Vadas, M. A. (1985) *Proc. Natl. Acad. Sci. U.S.A.* **82**, 8667–8671
16. Klebanoff, S. J., Vadas, M. A., Harlan, J. M., Sparks, L. H., Gamble, J. R., Agosti, J. M. & Waltersdorff, A. M. (1986) *J. Immunol.* **136**, 4220–4225
17. Roh, M. S., Moldawer, L. L., Ekman, L. G., Dinarello, C. A., Bistrian, B. R., Jeevanadam, M. & Brennan, M. F. (1986) *Metab. Clin. Exp.* **35**, 419–424
18. Tracey, K. J., Lowry, S. F., Beutler, B., Cerami, A., Albert, J. D. & Shires, G. T. (1986) *J. Exp. Med.* **164**, 1368–1373
19. Waage, A., Espevik, T. & Lamvik, J. (1986) *Scand. J. Immunol.* **24**, 739–743
20. Old, L. J. (1987) *Nature (London)* **326**, 330–331
21. Kettelhut, I. C., Fiers, W. & Goldberg, A. L. (1987) *Proc. Natl. Acad. Sci. U.S.A.* **84**, 4273–4277
22. Le, J. & Vilček, J. (1987) *Lab. Invest.* **56**, 234–248

Received 20 May 1987/15 July 1987; accepted 31 July 1987