Up-regulation of glucose metabolism in Kupffer cells following infusion of tumour necrosis factor

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Alterations of glucose metabolism and the oxidation of glutamine and palmitate were studied, by using specifically labelled substrates, in freshly isolated Kupffer cells and hepatic endothelial cells after infusion *in vivo* of human recombinant tumour necrosis factor- α (TNF; 7.5×10^5 IU/30 min per kg body wt., intravenously). Cells were incubated in a medium containing 5 mM-glucose, 0.4 mM-palmitate, 1 mM-lactate and 0.5 mM-glutamine. Administration of TNF *in vivo* increased glucose use in Kupffer cells by 70 %. Glucose oxidation in the tricarboxylic acid cycle and flux in the Embden–Meyerhof (EM) pathway were elevated by 40 and 80 % respectively. Treatment *in vitro* with 1 μ M-phorbol 12-myristate 13-acetate (PMA) resulted in a similar percentage increase in glucose use by Kupffer cells prepared from either saline- or TNF-treated rats. However, PMA increased the activity of the hexose monophosphate shunt (HMS) by 3- and 10-fold in cells isolated from saline- or TNF-infused animals respectively. A phagocyte stimulus *in vitro*, opsonized zymosan, increased glucose use by 30 % and doubled the flux through the HMS in Kupffer cells from saline-infused animals. The activity of the HMS in response to zymosan was increased by 400 % after TNF treatment. In endothelial cells, basal glucose utilization was not altered by TNF treatment. PMA increased HMS activity in endothelial cells to a similar degree after saline or TNF infusion. Zymosan, however, increased HMS activity only in endothelial cells from TNF-treated rats. Oxidation of palmitate or glutamine was not affected by TNF treatment either under basal conditions or after challenge *in vitro*. Our data indicate that, after phagocytosis *in vitro* or protein kinase C activation, glucose use and flux through the HMS increase in Kupffer cells. This is accompanied by increased glycolytic flux, with no changes in glucose oxidation in the tricarboxylic acid cycle. After TNF exposure, followed by a secondary stimulus, the enhanced glucose use by Kupffer cells is primarily channelled through the HMS pathway. These data suggest that the increased glucose use *in vivo* by Kupffer cells found after immune-stimulated conditions may subserve primarily the increased need for NADPH and HMS intermediates.

INTRODUCTION

In previous studies we demonstrated that, in response to bacterial lipopolysaccharides (Mészáros *et al.*, 1987a), various cytokines (Mészáros *et al.*, 1987b; Lang & Dobrescu, 1989) or phagocytic challenge *in vivo* (Spolarics *et al.*, 1991a), glucose use *in vivo* by immune-competent tissues is increased several-fold. In subsequent studies we verified that in the liver, one of the most responsive organs under these conditions, the non-parenchymal cells, in particular Kupffer cells and sequestered polymorphonuclear phagocytes, are largely responsible for the elevated glucose utilization (Mészáros *et al.*, 1991; Spolarics *et al.*, 1991a,b). Under these conditions, the exact metabolic fate of glucose or the oxidation of other potentially important exogenous substrates in hepatic non-parenchymal cells has not yet been elucidated (Brouwer *et al.*, 1988; Jones & Summerfield, 1988).

In endotoxaemia or sepsis, polymorphonuclear leucocytes and macrophages show increased phagocytic activity (Ogundottir & Weir, 1980). Ingestion of insoluble materials by these cells is accompanied by elevated consumption of oxygen and production of reactive oxygen metabolites (Badwey & Karnovsky, 1980). NADPH oxidase, which selectively utilizes NADPH as a co-enzyme, is responsible for the reduction of molecular oxygen and the generation of superoxide anions (Silverstein *et al.*, 1977). One of the sources of NADPH in these cells is glucose-6-phosphate dehydrogenase, which oxidizes glucose 6-phosphate to 3-oxo-6-phosphogluconate, producing two molecules of NADPH. It is generally accepted that superoxide production by phagocytizing macrophages and leucocytes is highly dependent on the avail-

ability of exogenous glucose (Sbarra & Karnovsky, 1959; Karnovsky *et al.*, 1975; Kiyotaki *et al.*, 1984; Mindham & Mayes, 1989). It has also been suggested that phagocytizing cells derive their energy by use of exogenous glucose (Griffin *et al.*, 1975). Other studies, however, have indicated that macrophages and other immune-competent cells readily utilize and oxidize glutamine and fatty acids to satisfy their energy requirements (Newsholme *et al.*, 1987; Ardawi, 1988; Newsholme & Newsholme, 1989).

The aim of the present study was to delineate the effect of administration *in vivo* of tumour necrosis factor- α (TNF), a cytokine known to mediate some of the metabolic effects of bacterial lipopolysaccharides (Mészáros *et al.*, 1987b; Bagby *et al.*, 1988; Beutler & Cerami, 1990), on exogenous substrate oxidation and glucose utilization in hepatic non-parenchymal cells. We evaluated the changes caused by TNF *in vivo* on the glucose flux through the hexose-monophosphate shunt (HMS), Embden–Meyerhof (EM) pathway and tricarboxylic acid cycle in Kupffer and hepatic endothelial cells. In addition, we examined the effect of TNF on substrate metabolism after functional challenge of these cells *in vitro*. We employed opsonized zymosan as a phagocytic stimulus *in vitro*, and stimulated the protein kinase C-dependent signal-transduction pathway by phorbol 12-myristate 13-acetate (PMA).

METHODS

Experimental animals

Male Sprague–Dawley rats (300–340 g; Charles River,

Abbreviations used: TNF, tumour necrosis factor- α ; HMS, hexose monophosphate shunt; EM, Embden–Meyerhof pathway; PMA, phorbol 12-myristate 13-acetate.

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Wilmington, MA, U.S.A.) were used for the study. Animals were fasted overnight with free access to water. The day before the experiments, catheters were placed in the jugular vein and carotid artery by using aseptic surgical procedures. The experiments were performed in accordance with the NIH guidelines for the use of animals.

Isolation of liver cells

Hepatic non-parenchymal cells were isolated by a modified version of the collagenase-perfusion method (Knook *et al.*, 1977). Briefly, after anaesthesia (9 mg of ketamine and 0.9 mg of xylazine/100 g body wt., intramuscularly), the liver was perfused *in situ* with Ca²⁺-free Hanks balanced salt solution at 37 °C under constant bubbling of gas (O₂/CO₂, 19:1) for 3 min. Then 0.025 % collagenase (Worthington Type I, 163 units/mg) and 0.001 % DNAase (Type II, 2540 Kunitz units/mg) were added and the liver was perfused for a further 4 min with Hanks balanced salt solution containing 1 mM-CaCl₂ and 0.5 % BSA (fraction V). After gentle shaking, the suspension was filtered and hepatocytes were sedimented at 50 g for 3 min. The remaining liver tissue was digested with 0.2 % Pronase E (Type XIV, 4.6 units/mg) containing 0.001 % DNAase for 10 min. Non-parenchymal cells from the two digestive treatments were collected and sedimented at 300 g for 10 min. The pellet of non-parenchymal liver cells was resuspended in 20 mM-Hepes/4.2 mM-bicarbonate solution (containing 120 mM-NaCl, 5.4 mM-KCl, 1.8 mM-CaCl₂, 1.2 mM-MgSO₄, 5 mM-glucose, 0.01 µg of DNAase/ml and 1 % BSA, pH 7.4) and subjected to centrifugal elutriation in a J2-21 centrifuge with J6B elutriator rotor (Beckman Instruments) (Figdon *et al.*, 1983). Endothelial cells were eluted at 2500 rev./min (23 ml/min), and Kupffer cells at 250 rev./min (49 ml/min). Kupffer cells were further purified by gradient centrifugation (Histopaque 1077) to separate Kupffer cells from contaminating polymorphonuclear cells (English & Andersen, 1974). Viability of cells assessed by Trypan Blue exclusion was over 98 % for the non-parenchymal cells. Purity of isolated cell preparations was over 96 % for endothelial and 94 % for Kupffer cells as identified by peroxidase-positivity (Wisse *et al.*, 1974) or after staining thin smears of cells suspensions with Wright-Giemsa Stain (EM Diagnostic Systems, Gibbstown, NJ, U.S.A.).

Incubation of liver cells

Freshly isolated Kupffer [(2–3) × 10⁶ cells/ml] or endothelial [(3–5) × 10⁶ cells/ml] cells were preincubated at 37 °C in Hepes/bicarbonate buffer containing 1 % BSA, 5 mM-glucose, 0.5 mM-glutamine, 1 mM-lactate and 0.4 mM-palmitate complexed with BSA as described previously (Montini *et al.*, 1981). After 20 min, 1 ml of cell suspension was mixed with 1 ml of incubation buffer containing tracers as follows: 2 µCi of [1-¹⁴C]glucose (55.9 mCi/mmol), 2 µCi of [6-¹⁴C]glucose (55.7 mCi/mmol), 6 µCi of [2-³H]glucose (21 Ci/mmol), 0.4 µCi of [U-¹⁴C]glutamine (260 mCi/mmol) or 1.6 µCi of [1-¹⁴C]palmitate (58 mCi/mmol). Palmitate was complexed with BSA. All radioactive materials were purchased from Amersham Corp., Arlington Heights, IL, U.S.A. Incubation was continued in stoppered silicone-treated vials for a further 60 min. Incubation was terminated by addition of 0.2 ml of 5 M-HClO₄. The liberated ¹⁴CO₂ was trapped in centre wells containing 0.2 ml methylbenzethonium hydroxide and counted for radioactivity in a Beckman scintillation spectrometer (LS 7500). ³H₂O produced by the cells was measured from the supernatant of the HClO₄-precipitated cell suspension as described previously (Zawalich *et al.*, 1977; Katz & Rognstad, 1976). Duplicate or triplicate incubations were analysed from each cell preparation.

Administration of TNF, PMA and zymosan

Human recombinant TNF-α (75 µg/kg body wt., activity 10⁷ units/mg; Cellular Products Inc., Buffalo, NY, U.S.A.) was dissolved in 0.5 ml of 0.9 % NaCl and infused intravenously in 30 min. Control animals received an equal volume of saline. PMA was dissolved in dimethyl sulphoxide and added to the cell suspensions at 1 µM. Zymosan was opsonized and administered at a concentration of 0.2 mg/10⁶ cells, as described by Bautista *et al.* (1990).

Unless otherwise mentioned, all chemicals and reagents were obtained from Sigma Fine Chemicals, St. Louis, MO, U.S.A.

Calculations of glucose fluxes

Distribution of utilized glucose in the HMS and EM pathway was calculated by the equation introduced by Katz & Wood (1963):

$$\frac{G1_{\text{CO}_2} - G6_{\text{CO}_2}}{1 - G6_{\text{CO}_2}} = \frac{3\text{HMS}}{1 + 2\text{HMS}}$$

where G1_{CO₂} and G6_{CO₂} are the specific yields of ¹⁴CO₂ from C-1- and C-6-labelled glucose. The specific yields of ¹⁴CO₂ were calculated by dividing the [1-¹⁴C]- or [6-¹⁴C]-glucose oxidation rate by the glucose detritiation rate determined on the same cell preparation. It was assumed that glucose is metabolized exclusively by the HMS and the EM pathway (Katz & Wood, 1963). In addition, we assumed that the detritiation rate of [2-³H]glucose is a direct indication of glucose utilization and that the hepatic non-parenchymal cells lack the capacity for gluconeogenesis (Crisp & Pogson, 1972; Katz & Rognstad, 1976; Van Berkel & Koster, 1977).

For statistical analysis of the data the one-way ANOVA was employed, followed by the Student-Newman-Keuls test.

RESULTS

The detritiation rate of [2-³H]glucose and oxidation rates of C-1 and C-6 in [1-¹⁴C]- and [6-¹⁴C]-glucose were measured in Kupffer cells (Table 1) after saline or TNF administration *in vivo* and after secondary stimulation *in vitro*. After intravenous infusion of TNF, the detritiation rate of [2-³H]glucose and the oxidation rate of C-6 in [6-¹⁴C]glucose were significantly elevated in Kupffer cells. Both the protein kinase C activating agent PMA and phagocytic challenge *in vitro* by opsonized zymosan caused a significant increase in the rate of oxidation of C-1 in [1-¹⁴C]glucose in Kupffer cells from saline-treated animals. Stimulation *in vitro* of Kupffer cells from TNF-infused animals, with either PMA or zymosan, caused a 2.5-fold larger increase in the oxidation rate of C-1 in [1-¹⁴C]glucose than that after saline infusion.

By using these tracers, it is possible to estimate the proportion of glucose flux through the HMS versus the EM pathway. The flux of glucose and the fraction of utilized glucose metabolized via the HMS and EM pathways are also shown in Table 1. In Kupffer cells, TNF treatment increased the glucose flux via the EM pathway by about 100 % without altering the flux via the HMS as compared with values obtained after saline treatment. At the same time, the percentage share of the EM in glucose utilization was similar in Kupffer cells from saline and TNF-infused rats. PMA increased glucose flux via the HMS by about 150 % in Kupffer cells from saline-treated rats. After TNF treatment, in response to PMA, a 10-fold increase was found in the glucose flux through the HMS. Interestingly, PMA administration failed to cause a significant increase in the glucose flux via the EM pathway after either saline or TNF; the percentage share of the EM decreased. Phagocytic challenge of Kupffer cells *in vitro* by opsonized zymosan tended to produce

Table 1. Effect of TNF administration *in vivo* on the catabolism of glucose by Kupffer cells

Kupffer cells isolated from rats treated *in vivo* with saline or TNF were incubated in the presence of various glucose tracers, and the proportions of glucose flux through the HMS and the EM pathway were determined as described in the Methods section. Results are means $\pm$ S.E.M. ($n = 8-10$). Units: ^anmol of glucose/h per mg of cell protein recovered in ³H₂O or ¹⁴CO₂; ^bpercentage distribution of utilized glucose in the pathways; ^cnmol of glucose/h per mg of cell protein metabolized in the pathways. *Statistically significant differences of the PMA and zymosan treatments *in vitro* from vehicle ($P < 0.05$). †Statistically significant difference of the TNF treatment *in vivo* from saline ($P < 0.05$).

Treatment <i>in vivo</i>	Treatment <i>in vitro</i>		
	Vehicle	PMA	Zymosan
Tracer used			
[2- ³ H]Glucose ^a			
Saline	34.3 $\pm$ 3.6	45.6 $\pm$ 4.1	42.1 $\pm$ 5.2
TNF	59.4 $\pm$ 4.4†	86.6 $\pm$ 6.8*†	77.0 $\pm$ 8.1*†
[1- ¹⁴ C]Glucose ^a			
Saline	7.7 $\pm$ 0.8	17.0 $\pm$ 1.7*	14.0 $\pm$ 2.5*
TNF	7.6 $\pm$ 0.7	43.3 $\pm$ 4.3*†	26.1 $\pm$ 2.5*†
[6- ¹⁴ C]Glucose ^a			
Saline	0.9 $\pm$ 0.04	1.0 $\pm$ 0.08	1.0 $\pm$ 0.07
TNF	1.3 $\pm$ 0.07†	1.5 $\pm$ 0.06†	1.3 $\pm$ 0.04†
HMS pathway			
Saline			
(% of uptake) ^b	8.6 $\pm$ 0.8	20.6 $\pm$ 2.3*	12.7 $\pm$ 1.2
(nmol/h per mg) ^c	2.8 $\pm$ 0.3	8.1 $\pm$ 0.9*	5.9 $\pm$ 1.3
TNF			
(% of uptake) ^b	4.7 $\pm$ 0.5	27.2 $\pm$ 1.8*†	18.6 $\pm$ 1.7*†
(nmol/h per mg) ^c	2.4 $\pm$ 0.3	23.1 $\pm$ 2.6*†	11.6 $\pm$ 1.2*†
EM pathway			
Saline			
(% of uptake) ^b	91.4 $\pm$ 0.8	79.4 $\pm$ 2.4*	87.2 $\pm$ 1.1
(nmol/h per mg) ^c	31.5 $\pm$ 3.3	37.6 $\pm$ 3.7	36.2 $\pm$ 4.2
TNF			
(% of uptake) ^b	95.3 $\pm$ 0.5	72.7 $\pm$ 1.6*†	81.4 $\pm$ 1.6*†
(nmol/h per mg) ^c	57.0 $\pm$ 4.3†	63.6 $\pm$ 5.5†	65.3 $\pm$ 7.7†

changes in glucose metabolism similar to those observed in the presence of PMA. The increase in the activity of HMS in response to zymosan, however, was statistically significant only after TNF treatment.

In the endothelial cells (Table 2), TNF infusion did not cause any changes in the detritation and oxidation rates of C-1 and C-6 of glucose. PMA treatment increased the rate of [2-³H]glucose detritation and oxidation of C-1 in [1-¹⁴C]glucose to a similar degree after saline or TNF infusion. Zymosan addition to endothelial cells resulted in a slight increase in oxidation of C-1 in [1-¹⁴C]glucose only after TNF treatment. TNF administration did not cause significant changes in the basal flux or distribution of glucose in the HMS and EM pathways in endothelial cells. PMA increased glucose flux via both the HMS and EM pathways. The increment of glucose flux via the HMS in response to PMA was very similar after TNF or saline treatment *in vivo*. It was also demonstrated that, although HMS activity was not altered by phagocytosis *in vitro* in endothelial cells prepared from saline-treated animals, it was slightly but significantly increased after TNF treatment.

In another series of experiments we determined whether TNF treatment *in vivo*, by itself or in combination with PMA or zymosan challenge *in vitro*, influenced the oxidation rate of glutamine and palmitate by isolated non-parenchymal cells. Table 3 shows that Kupffer and hepatic endothelial cells readily

Table 2. Effect of TNF administration *in vivo* on the catabolism of glucose by hepatic endothelial cells

For conditions and symbols see the legend to Table 1.

Treatment <i>in vivo</i>	Treatment <i>in vitro</i>		
	Vehicle	PMA	Zymosan
Tracer used			
[2- ³ H]Glucose ^a			
Saline	18.7 $\pm$ 1.3	30.7 $\pm$ 1.7*	21.0 $\pm$ 1.7
TNF	21.0 $\pm$ 1.7	33.0 $\pm$ 1.9*	24.1 $\pm$ 2.2
[1- ¹⁴ C]Glucose ^a			
Saline	4.3 $\pm$ 0.3	7.2 $\pm$ 0.6*	4.1 $\pm$ 0.4
TNF	3.8 $\pm$ 0.3	7.9 $\pm$ 0.7*	7.0 $\pm$ 0.7*†
[6- ¹⁴ C]Glucose ^a			
Saline	1.0 $\pm$ 0.07	1.1 $\pm$ 0.06	1.1 $\pm$ 0.08
TNF	1.1 $\pm$ 0.08	1.1 $\pm$ 0.07	1.2 $\pm$ 0.08
HMS pathway			
Saline			
(% of uptake) ^b	7.1 $\pm$ 0.6	8.6 $\pm$ 0.9	5.7 $\pm$ 0.7
(nmol/h per mg) ^c	1.3 $\pm$ 0.1	2.5 $\pm$ 0.2*	1.2 $\pm$ 0.1
TNF			
(% of uptake) ^b	5.6 $\pm$ 0.5	9.2 $\pm$ 0.9*	10.7 $\pm$ 1.0*†
(nmol/h per mg) ^c	1.1 $\pm$ 0.09	2.9 $\pm$ 0.4*	2.6 $\pm$ 0.3*†
EM pathway			
Saline			
(% of uptake) ^b	92.9 $\pm$ 0.6	91.4 $\pm$ 0.9	94.3 $\pm$ 0.7
(nmol/h per mg) ^c	17.4 $\pm$ 1.2	28.2 $\pm$ 1.7*	19.8 $\pm$ 1.7
TNF			
(% of uptake) ^b	94.4 $\pm$ 0.5	90.8 $\pm$ 1.0*	89.3 $\pm$ 0.9*†
(nmol/h per mg) ^c	20.0 $\pm$ 1.6	30.1 $\pm$ 1.7*	21.6 $\pm$ 2.2

Table 3. Effect of TNF administration *in vivo* on glutamine and palmitate oxidation by Kupffer and hepatic endothelial cells

Substrate oxidation rates by the isolated cells were measured as described in the Methods section. Values (nmol of substrate/h per mg of cell protein) are means $\pm$ S.E.M. ($n = 6$).

Cells	Treatment <i>in vivo</i> and tracer used	Treatment <i>in vitro</i>		
		Vehicle	PMA	Zymosan
Kupffer	[U- ¹⁴ C]Glutamine			
	Saline	4.4 $\pm$ 0.7	4.7 $\pm$ 0.8	4.7 $\pm$ 0.8
	TNF	4.8 $\pm$ 0.9	5.1 $\pm$ 0.9	5.1 $\pm$ 0.9
	[1- ¹⁴ C]Palmitate			
Endothelial	Saline	0.9 $\pm$ 0.2	1.0 $\pm$ 0.1	0.8 $\pm$ 0.2
	TNF	1.0 $\pm$ 0.2	1.1 $\pm$ 0.2	1.2 $\pm$ 0.2
	[U- ¹⁴ C]Glutamine			
	Saline	6.0 $\pm$ 0.4	6.0 $\pm$ 0.3	6.1 $\pm$ 0.3
	TNF	6.7 $\pm$ 0.6	6.9 $\pm$ 0.6	7.0 $\pm$ 0.6
	[1- ¹⁴ C]Palmitate			
	Saline	1.2 $\pm$ 0.3	1.2 $\pm$ 0.2	1.1 $\pm$ 0.3
	TNF	1.3 $\pm$ 0.4	1.4 $\pm$ 0.4	1.4 $\pm$ 0.3

oxidize glutamine and palmitate. It is also shown that TNF treatment did not significantly change the rate of oxidation of either substrate. Furthermore, administration of PMA or zymosan *in vitro* did not result in any significant alteration in the oxidation rates of these substrates.

DISCUSSION

TNF is an important member of the cytokine cascade mediating the metabolic changes observed in sepsis or endotoxaemia (Mészáros *et al.*, 1987b; Bagby *et al.*, 1988; Spolarics

et al., 1991b). TNF has been shown to up-regulate different cell functions, including increased superoxide production and phagocytic activity of leucocytes and macrophages (Beutler & Cerami, 1990). The present study elucidates some of the effects of a brief infusion *in vivo* of a non-lethal dose of TNF on the intermediary metabolism of hepatic non-parenchymal cells.

TNF administration *in vivo* results in characteristic changes in glucose metabolism, primarily in Kupffer cells. After TNF treatment, glucose uptake, glycolysis and the oxidation of glucose in the tricarboxylic acid cycle are considerably elevated, which is sustained during the isolation procedure and incubation. This suggests that one possible effect of TNF is the stimulation of the activity and/or number of glucose transporters. Our findings are consistent with the increased expression of mRNA for glucose transporters demonstrated after short-term TNF or endotoxin administration (Cornelius *et al.*, 1989; Lee *et al.*, 1987). We assume that the increased oxidation of glucose in the tricarboxylic acid cycle is a consequence of an elevated glucose entry into the cells. This is supported with the findings that the oxidation of glutamine and palmitate was not increased after TNF infusion. It is also possible, however, that TNF treatment results in an increased activity of the pyruvate dehydrogenase.

Another effect that TNF has on Kupffer cells is the ability to prime these cells for the subsequent effect of secondary stimuli. Thus, although TNF alone did not significantly alter the flux of glucose through the HMS, it was markedly elevated after phagocytic stimulus or activation of protein kinase C. It is noteworthy that upon PMA or zymosan treatment the increased glucose flux in the HMS is not accompanied by markedly elevated glycolytic flux or oxidation of glucose in the tricarboxylic acid cycle in Kupffer cells. These observations indicate that the utilization of glucose and its catabolism via the HMS is increased selectively by the secondary stimuli. A similar selective effect of granulocyte-monocyte-colony-stimulating factor on glucose metabolism was demonstrated in thymocytes (Rist *et al.*, 1990). One of the potential explanations for the increased glucose flux via the HMS is that the TNF-induced glucose transport is no longer rate-limiting in supplying glucose for the pathway. The increased activity of HMS may simply be due to the increased consumption of NADPH, thus increasing the NADP⁺/NADPH ratio upon secondary stimuli. Our findings may also be interpreted as suggesting that the utilization of glucose and the activity of the HMS are co-ordinately regulated by protein kinase C-dependent mechanisms. Thus TNF may prime the signal-transduction system, which may lead to an increased response of the HMS activity upon demand.

The rate of glycolysis in Kupffer cells reported in this study is lower than that found in peritoneal macrophages (Newsholme & Newsholme, 1989; Newsholme *et al.*, 1987). This suggests that the aerobic oxidative metabolism is more important in the basal energy requirements for the resident macrophages of the liver (Spolarics *et al.*, 1991c). The percentage contribution of the HMS to glucose utilization by Kupffer cells is in good agreement with values found on whole liver tissue (Wood, 1985; Williams, 1980).

The rate of glucose utilization by Kupffer cells is about twice that found in endothelial cells under basal conditions when compared on a cell-protein basis. The proportions of glucose fluxes through different pathways are very similar in Kupffer and endothelial cells prepared from controls rats. However, the capacity of the HMS is higher in Kupffer than in endothelial cells, as indicated by the larger response of Kupffer cells after protein kinase C activation or phagocytic stimuli. It is noteworthy that the effects of TNF on the glucose metabolism by hepatic endothelial cells were much smaller than found in Kupffer cells, although the directional changes tended to be similar.

The oxidation of C-1- and C-6-labelled glucose and the C-1/C-6 ratios have been used for the estimation of HMS activity. However, the quantitative conclusions from the oxidation rates of C-1/C-6 ratios may be subject to errors (Labarre, 1989). In the present study we used calculations introduced by Katz & Wood (1963) for the evaluation of glucose flux through the main catabolic pathways. For the determination of glucose utilization, which is necessary for the calculations of specific ¹⁴CO₂ yields from C-1- and C-6-labelled glucose, the [2-³H]glucose-detritiation technique was used. Formation of ³H₂O from [2-³H]glucose is a reliable measure of glucose utilization in tissues lacking mechanisms of active glucose recycling (Katz & Rognstad, 1976). However, a proportion of the ³H in position 2 of glucose can be retained when glucose enters the HMS (Katz & Rognstad, 1969). This may result in a possible underestimation of glucose utilization, which could modify our results concerning the proportion of HMS under our experimental conditions. As illustrated in Fig. 1, the magnitude of this error in estimating the glucose flux through HMS depends on the contribution of the shunt. However, even when glucose utilization is underestimated by as much as 50 %, the error in the glucose flux via the HMS is less than 15 %. This indicates that the method used in our experiments is sufficiently reliable for the quantitative measurement of the flux of glucose through major pathways.

Earlier studies have indicated the importance of glutamine utilization and oxidation, as well as fatty acid oxidation, in the energy metabolism of macrophages and other immune-competent cells (Newsholme *et al.*, 1987; Ardawi, 1988; Newsholme & Newsholme, 1989; Caldwell, 1989). We previously demonstrated that Kupffer and hepatic endothelial cells also readily oxidize glutamine and fatty acids (Spolarics *et al.*, 1991c). In the present study we found that TNF did not alter the oxidation of these substrates in either Kupffer or endothelial cells. At the same time, TNF increased glycolytic flux and the glucose oxidation in the tricarboxylic acid cycle in Kupffer cells. These indicate that the augmented energy metabolism of Kupffer cells after TNF treatment depends primarily on the utilization of glucose rather than the oxidation of other available exogenous substrates. The increased glucose utilization after TNF could account for a 10–15 % enhancement in ATP synthesis generated from exogenous substrates provided for the cells in our exper-

Fig. 1. Calculated error of the glucose flux through the HMS as a function of the underestimation of glucose utilization

Zero to 50 % underestimation of glucose utilization was assumed in Kupffer cells from saline and TNF-treated animals, under basal conditions and after PMA challenge, as indicated. Flux of glucose through the HMS was calculated and expressed as percentage change of initial value. Key: ·····, saline + vehicle; —, TNF + vehicle; — x —, saline + PMA; ---, TNF + PMA.

iments (Spolarics *et al.*, 1991c). The unaltered glycolytic flux and glucose oxidation in the presence of zymosan are in agreement with the observations that phagocytosis is not highly dependent on newly synthesized ATP (Silverstein *et al.*, 1977). This may indicate a potential role of an increased phosphocreatine turnover during phagocytosis, as was demonstrated in elicited peritoneal macrophages (Loike *et al.*, 1979).

Our results indicate the importance of the increased glucose utilization by the HMS under immune-stimulated conditions. The most probable function of the enhanced glucose metabolism in the HMS is to provide intermediates of the shunt and NADPH for the cells. NADPH can be utilized in synthetic processes and/or the production of reactive oxygen metabolites. According to our present study, the NADPH that can be synthesized by the oxidation of glucose in the HMS after PMA or zymosan exposure of TNF-treated Kupffer cells may be sufficient to produce O_2^- at a rate of 25–50 nmol/h per mg of cell protein. Under similar conditions, after TNF or lipopolysaccharide administration, *in vivo*, Kupffer cells produced O_2^- at a rate of 30–60 nmol/h per mg of cell protein in response to zymosan or PMA treatment (Bautista *et al.*, 1990). These values correlate well and suggest the pivotal importance of elevated glucose utilization in the generation of reactive oxygen metabolites under an immune-stimulated condition.

The effect of TNF on glucose metabolism by Kupffer cells is similar to that demonstrated on different cell lines after granulocyte-monocyte-colony-stimulating factor or interleukin-1 administration (Rist *et al.*, 1990; Phillips & Hamilton, 1989). These findings raise the possibility of a synergistic effect of these cytokines on the intermediary metabolism of immune-competent cells, despite their diverse effects on other cell functions. This suggests that cytokines released from a variety of cells may be responsible for the sustained metabolic up-regulation, increased glucose utilization and superoxide production of immune-competent cells in sepsis or endotoxaemia.

This work was supported by NIH Grant GM 32654. Thanks are due to June T. Bechtel and Jean Carnal for their excellent technical assistance.

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Received 21 February 1991/25 April 1991; accepted 8 May 1991