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Comparison of TNF-Mediated Glucose Catabolism between the TNF-Sensitive and -Resistant Cell Lines

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When murine fibrosarcoma L929 cells, a TNF-sensitive cell line, were treated with recombinant human tumor necrosis factor-α (rhTNF-α), the activities of glycolytic regulatory enzymes and lactate dehydrogenase increased up to 100~150% compared to the control L929 cells after TNF treatment. By using various metabolic inhibitors and activators, it was found that cAMP-dependent protein kinase is responsible for the increase of activities of the glycolytic enzymes. The activities of glycolytic regulatory enzymes and lactate dehydrogenase of TNF-resistant A549 cells, a human lung carcinoma cell line, did not increase significantly compared to TNF-sensitive L929 cells upon TNF treatment. In contrast, the pyruvate carboxylase activities of A549 cells, but not L929 cells, increased up to 30~40% after TNF treatment. The data suggest that pyruvate carboxylase activity may contribute to the compensation of energy loss mediated by TNF treatment in TNF-resistant A549 cells.

Keywords: cAMP, Glucose uptake, Glycolytic enzyme, Lactate dehydrogenase, Pyruvate carboxylase, Tumor necrosis factor.

Introduction

Tumor necrosis factor (TNF-α) was originally described as a component found in the serum of animals injected with lipopolysaccharide (LPS) that could mediate the necrosis of transplanted tumors *in vivo* (Carswell *et al.*, 1975). This cytokine is produced predominantly by activated macrophages (Lee *et al.*, 1989; Baik *et al.*, 1995) and has many activities in addition to tumor cell cytotoxicity. For example, TNF influences a variety of biological processes including mitogenesis, differentiation, angiogenesis, and immunoregulation (Beutler and Cerami, 1989; Grunfeld and Feingold *et al.*, 1991).

In vitro, TNF is cyototoxic to certain tumor cell lines but not to normal cells (Carswell *et al.*, 1975; Sugarman *et al.*, 1985). However, the mechanism of this selective cytotoxicity is still unclear. Several modes of cell killing have been suggested, such as DNA fragmentation (Dealtry *et al.*, 1987), alteration in arachidonic acid metabolism (Suffys *et al.*, 1987), serine protease involvement (Voelkel-Johnson *et al.*, 1995), oxidative stress (Zimmerman *et al.*, 1989; Hennet *et al.*,1992), and depletion of ATP/NADH stores (Schulze-Osthoff *et al.*, 1992). Among the reported effects of TNF on target cells, oxidative stress in mitochondria may be the common denominator of these events. In this regard, antioxidants have been reported to confer some protection against TNF cytotoxicity (Matthews *et al.*,1987; Satomi *et al.*,1988), and TNF-resistant L929 cells had a decreased ability to produce superoxide anion in response to TNF (Polla *et al.*,1996). The authors of this paper as well as other researchers have already observed that overexpression of MnSOD increased resistance to TNF (Wong *et al.*,1989; Kim and Kim, 1997; 1999).

Matthews originally suggested that cells might attempt to compensate for the mitochondrial dysfunction with an increased rate of glycolysis (Matthews, 1983). Others have also reported that TNF enhanced glucose metabolism and lactate production in various cell types (Evans *et al.*,1989; Naichen *et al.*,1995).

In this present study, the activities of glycolytic regulatory enzymes, lactate dehydrogenase, and pyruvate carboxylase in TNF-sensitive L929 cells and TNF-resistant A549 cells were examined and compared after TNF treatment. This study suggests that the increase of pyruvate carboxylase activity in A549 cells after TNF treatment may be another parameter for cellular resistance against TNF cytotoxicity.

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Materials and Methods

Materials Recombinant human TNF-α was produced in *Escherichia coli* and purified to approximately 90% homogeneity as described previously (Choeng and Kim, 1994). The preparation had a specfic activity of 4×10^6 units/mg of protein. Minimal essential medium (MEM), HBSS (Hank's balanced solution), trypsin, and fetal bovine serum (FBS) were purchased from Gibco (Grand Island, USA). N-[2-(methylamino)ethyl]-5-isoquinolinesulfonamide (H-8) was obtained from Seikagaku Co. (Tokyo, Japan). Staurosporine, indomethacin, phorbol 12-myrystate13-acetate (PMA), phenylmethyl sulfonyl fluoride (PMSF), Trizmabase, and Trizma hydrochloride were purchased from Sigma Chemical Co. (St. Louis, USA). All chemicals for enzyme assays were purchased from Boehringer Mannheim (Mannheim, Germany).

Cell culture Murine fibrosarcoma L929 cells and human lung carcinoma A549 cells were obtained from the American Type Culture Collection (Rockville, USA). The cells were grown in minimal essential medium (MEM) supplemented with 10% FBS and maintained at 37°C and 5% CO_2.

Cytotoxicity assay TNF activity was determined by an assay for the killing of L929 cells as described by Flick and Gifford (1984). One unit of TNF activity was determined as the amount required for 50% cell lysis.

Assay for glycolytic enzymes L929 or A549 cells were plated at a density of 2×10^6 cells in a 25-cm^2 flask. After 12 h, cells were incubated in MEM containing the recombinant human TNF-α for 2, 4, 6, 8, 10, 12, and 24 h. L929 cells were treated with 10 and 100 units/ml of TNF-α and A549 cells were treated with 10 and 250 units/ml of TNF-α. Incubated cells were harvested by centrifugation, washed with phosphate-buffered saline, pH 7.4, and disrupted by sonication at 4°C. Lysates in 20 mM Tris·HCl and 1 mM phenylmethyl sulfonyl fluoride (PMSF) were centrifuged at 15,000 × *g* for 5 min at 4°C and then supernatants were added to the reaction mixture (total volume, 1.0 ml) for the assay of glycolytic enzyme activity. Enzyme activities were determined by the following methods: Hexokinase as described by Bergmeyer *et al.* (1983), 6-phosphofructo-1-kinase by Crane and Sols (1955), and pyruvate kinase by Fuji and Miwa (1983).

Assay for lactate dehydrogenase and pyruvate carboxylase L929 or A549 cells were plated at a density of 5×10^5 cells in 6-well plates. After 12 h, cells were incubated in a medium containing various units of recombinant human TNF-α for 2, 4, 6, 8, 10, 12, and 24 h. L929 cells were treated with 10 and 100 units/ml of TNF-α and A549 cells were treated with 10 and 250 units/ml of TNF-α. Incubated cells were harvested by centrifugation, washed with phosphate-buffered saline, pH 7.4, and disrupted by lysis buffer containing 20 mM Tris·HCl buffer (pH 7.5) and 0.2% TritonX-100. Lysates were centrifuged at 15,000 × *g* for 5 min at 4°C and supernatants were then used for enzyme assays. Activities of lactate dehydrogenase and pyruvate carboxylase were measured by the methods of Vassault (1983) and McClure *et al.* (1971), respectively.

Measurement of intracellular cAMP Cells (2×10^5 ml^{-1}) grown in a culture flask (25 cm^2) were washed once with PBS and treated with TNF-α (1, 10, 100 units/ml) for 15 min, 1, 2, 4, and 8 h. Cells were harvested and then resuspended in HBSS and 65% ice-cold ethanol, and finally centrifuged at 2,000 × *g* for 15 min at 4°C. cAMP enzyme-immunoassay system kits (Amersham, Arington Heights, USA) were used to measure the cAMP concentrations of precipitates.

Glucose uptake Glucose uptake was measured using a modification of White *et al.* (1981). Cells (10^5 ml^{-1}) grown in a culture flask (25 cm^2) were washed once with PBS and treated with TNF-α (50 units/ml) for 4, 8, 12, 18, and 24 h. Cells were washed with serum-free MEM and incubated with serum-free MEM containing 0.3 μCi 2-deoxy-[^{3}H] glucose (Amersham, Arlington Heights, USA, 16 Ci/mmol) for 30 min. After washing twice with PBS, 1 ml of 0.4 M NaOH was added to lyse the cells and the lysate was incubated for 1 h at 37°C. Three hundred μl of the lysate was taken for scintillation counting. Protein concentration was measured by the Bradford method (Bradford, 1976).

Statistical analysis All data are presented as mean ± SD, and were determined from at least three individual experiments.

Results

Effects of TNF treatment on glycolytic enzymes activities The activities of glycolytic enzymes (hexokinase, 6-phosphofructo-1-kinase, and pyruvate kinase) in TNF-sensitive L929 cells and TNF-resistant A549 cells were measured in the absence or presence of TNF (10, 100, and 250 units/ml) for 2, 4, 6, 8, 10, 12, and 24 h. As shown in Fig. 1, the relative activity of hexokinase in L929 cells increased approximately up to 100% after treatment with 100 units/ml of TNF when compared with TNF-untreated control cells (Fig. 1A). Upon treatment with 100 units/ml of TNF, the activity of 6-phosphofructo-1-kinase, a key regulatory enzyme in glycolysis, also increased up to 150% of the control activity (Fig. 1C). The activity of pyruvate kinase increased approximately 70% compared to the control at 6 h following treatment with 100 units/ml of TNF treatment and then gradually decreased to control levels at a later stage (Fig. 1F). When L929 cells were treated with 10 units/ml of TNF, the activity of hexokinase increased up to 15~45% (Fig. 1A) and the activity of 6-phosphofructo-1-kinase increased approximately 50~150% compared to that of the control L929 cells (Fig. 1C). The activity of pyruvate kinase in TNF-treated L929 cells increased 10~50% compared to that of the control L929 cells (Fig. 1E).

In A549 cells, a TNF-resistant cell line, the activities of hexokinase and pyruvate kinase, after treatment with 250 units/ml of TNF, increased only 10% to 20% compared to those of the control A549 cells (Figs. 1B and 1F). However, the activity of 6-phosphofructo-1-kinase increased approximately 50% at 10 h in TNF-treated A549

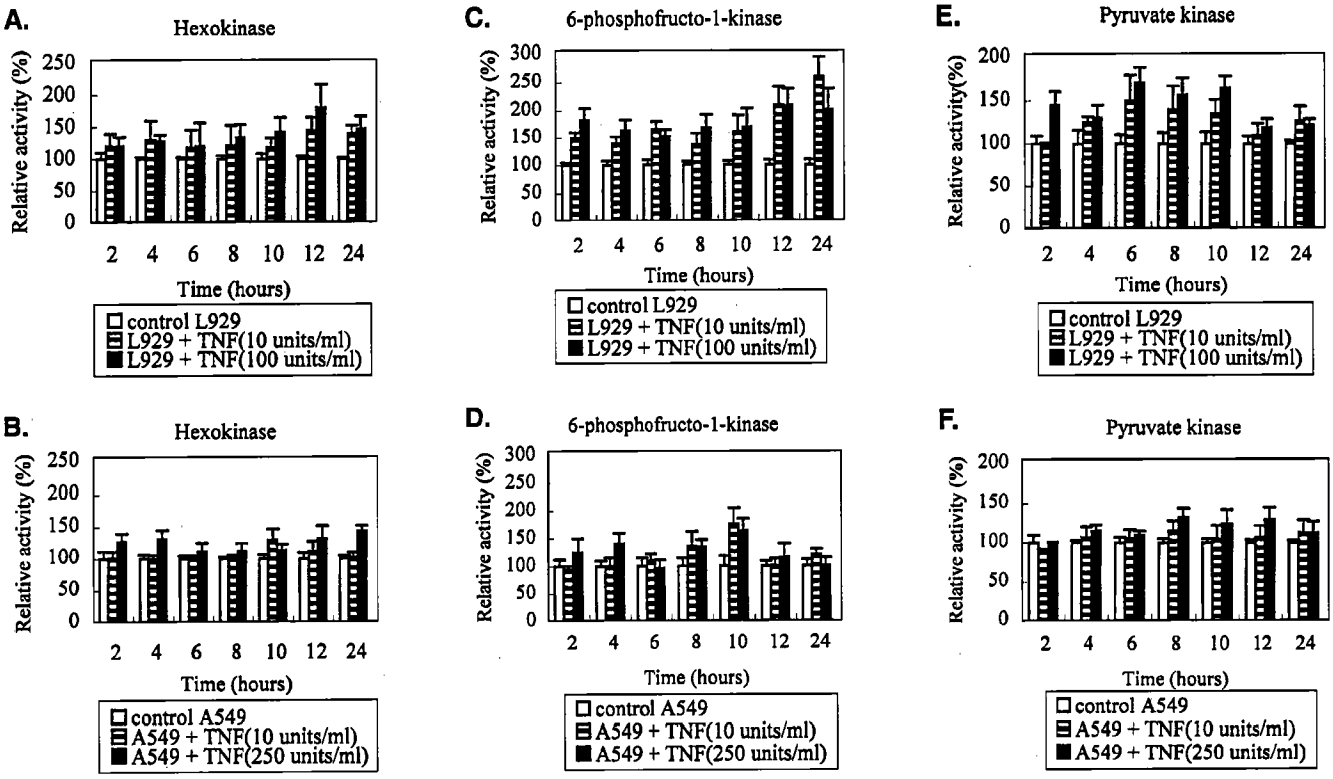


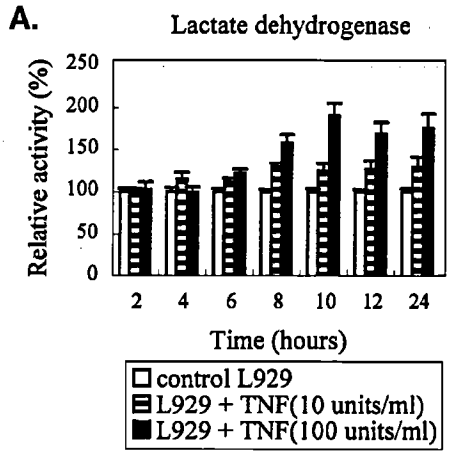
Fig. 1. Relative activities (%) of glycolytic enzymes of L929 and A549 in the presence or absence of TNF-α. Relative activity (%) represents the specific activity of the TNF-treated L929 and A549 cells compared to the specific activity of the TNF-untreated L929 and A549 cells, respectively. The specific activities of TNF-untreated L929 and A549 cells were both set to 100% activity. Values are presented as mean ± SD of four independent experiments. A, B, hexokinase; C, D, 6-phosphofructo-1-kinase; E, F, pyruvate kinase.

cells compared to that of the control A549 cells (Fig. 1D). When A549 cells were treated with 10 units/ml of TNF, the activities of hexokinase and pyruvate kinase did not increase compared to those of the control A549 cells (Figs. 1B and 1F). Upon treatment with 10 units/ml of TNF, the activity of 6-phosphofructo-1-kinase in A549 cells increased approximately 60% at 10 h and did not increase at 2, 4, 6, 8, 12, and 24 h compared to that of the control A549 cells (Fig. 1D)

These data suggest that 6-phosphofructo-1-kinase is the most sensitively affected enzyme by TNF treatment and low glycolytic activities may be a common parameter indicating cellular resistance against TNF cytotoxicity.

Effects of TNF treatment on lactate dehydrogenase activity The levels of lactate dehydrogenase activity in TNF-treated L929 cells increased in a time-dependent manner. The lactate dehydrogenase activity in L929 cells increased up to 100% at 10 h after treatment with 100 units/ml of TNF compared to those of the control L929 cells and then maintained very high levels until 24 h (Fig. 2A). Upon treatment with 10 units/ml of TNF, the levels of lactate dehydrogenase activity in TNF-treated L929 cells increased approximately 30% compared to those of the control L929 cells (Fig. 2A). However, there were no significant changes in lactate dehydrogenase activities either with or without TNF treatment in A549 cells (Fig. 2B).

Pyruvate carboxylase activity in L929 and A549 cells after TNF treatment As shown in Fig. 3A, the activity of pyruvate carboxylase in L929 cells incubated with TNF (10 and 100 units/ml) for 24 h was the same as for the control L929 cells. In contrast, the levels of pyruvate carboxylase activities in TNF-treated A549 cells were 30 to 40% higher than those of TNF-untreated A549 cells after treatment with 250 units/ml of TNF (Fig. 3B). Upon treatment with 10 units/ml of TNF, the levels of pyruvate carboxylase activity of TNF-treated A549 cells increased 10~30% compared to those of the control A549 cells (Fig. 3B). To determine whether the activation of pyruvate carboxylase in A549 cells would lead to gluconeogenesis, the activity of phosphoenolpyruvate carboxykinase was examined and compared with that of L929 cells. There was no difference in phosphoenolpyruvate carboxykinase activity between L929 and A549 cells (data not shown). This data could be interpreted to mean that TNF-resistant A549 cells might be able to operate the TCA cycle



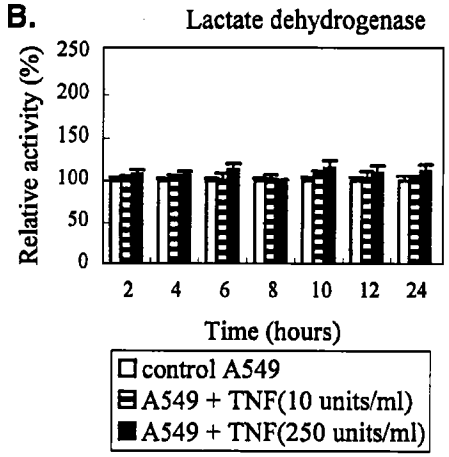
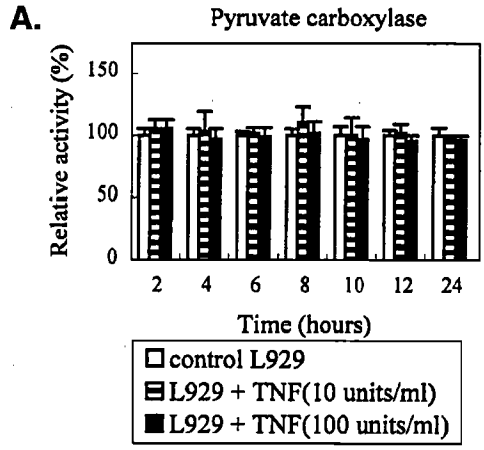


Fig. 2. Relative activities (%) of lactate dehydrogenase of L929 and A549 cells in the presence or absence of TNF-α. Relative activity (%) represents the specific activity of the TNF-treated L929 and A549 cells compared to the specific activity of the TNF-untreated L929 and A549 cells, respectively. The specific activities of TNF-untreated L929 and A549 cells were set to 100% activity. Values are presented as mean ± SD of three independent experiments.



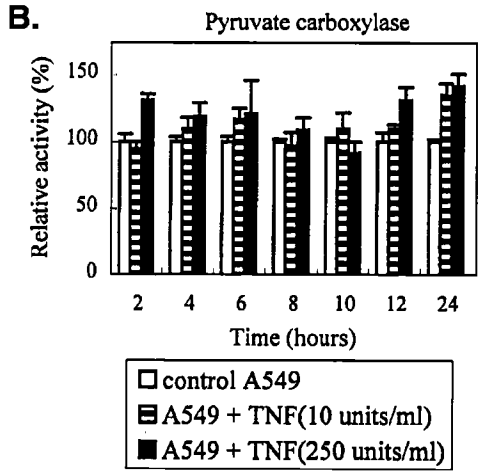


Fig. 3. Relative activities (%) of pyruvate carboxylase of L929 and A549 cells in the presence or absence of TNF-α. Relative activity (%) represents the specific activity of the TNF-treated L929 and A549 cells compared to the specific activity of the TNF-untreated L929 and A549 cells, respectively. The specific activities of TNF-untreated L929 and A549 cells were both set to 100% activity. Values are presented as mean ± SD of three independent experiments.

properly, even in the presence of TNF, due to their normal lactate dehydrogenase and increased pyruvate carboxylase activities.

Signal transduction in the TNF-mediated increase of glycolysis The increased activity of 6-phosphofructo-1-kinase in L929 cells after TNF treatment was reduced to the level of TNF-untreated L929 cells when L929 cells were incubated with 10 μM H-8, an inhibitor of cyclic-nucleotide-dependent protein kinase (Takuma, 1988) (Fig. 4). For pyruvate kinase, the activity was also reduced to the level of control L929 cells upon TNF treatment in the presence of H-8 (data not shown). PMA, which is known as an effector of protein kinase C (Mauduit *et al.*,1989), did not affect 6-phosphofructo-1-kinase activity increased by TNF treatment (Fig. 4). Indomethacin, an arachidonic acid metabolism inhibitor (Hori *et al.*, 1989), also did not have any effect on the TNF-mediated increase of 6-phosphofructo-1-kinase activity in the same experiment (Fig. 4).

We also examined the amount of intracellular cAMP and glucose uptake in cells after TNF treatment. As shown in Fig. 5, the amount of intracellular cAMP increased up to 50% compared to the control L929 levels at 2 h following TNF treatment. Glucose uptake in L929 cells almost tripled at 4 h after TNF treatment and then maintained relatively high levels up to 8 h (Fig. 6). Taken together, these data suggest that the increase of glycolysis by TNF treatment in L929 cells may be mediated by cAMP-dependent protein kinase A.

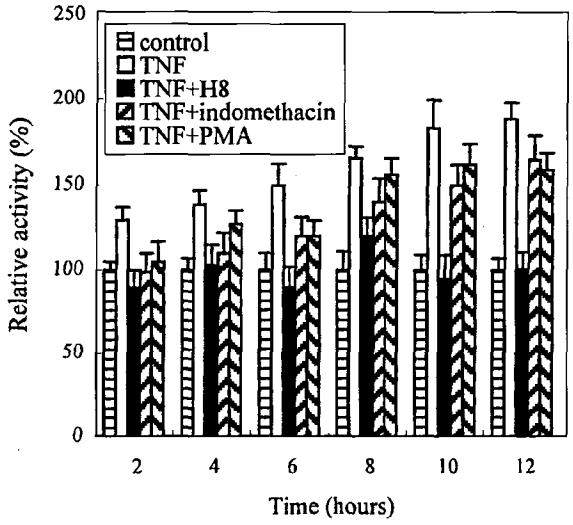


Fig. 4. Effect of H-8 (10 μM), PMA (10 μM), and indomethacin (200 μM) on 6-phosphofructo-1-kinase activity of L929 cells in the presence or absence of TNF-α (100 units/ml). Values are presented as mean ± SD of three independent experiments.

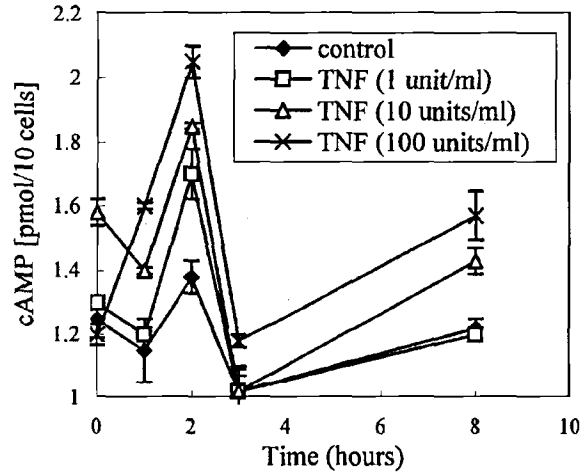


Fig. 5. cAMP production of L929 cells in the presence or absence of TNF-α (1, 10, 100 units/ml). Values are presented as mean ± SD of three independent experiments.

Discussion

The participation of mitochondria in TNF-induced cytotoxicity was inferred from the observation that these organelles were altered in target cells soon after treatment with TNF (Schulze-Osthoff *et al.*, 1992). It has been demonstrated that mitochondrial production of oxygen radicals mainly generated at the ubisemiquinone site is a casual mechanism of TNF cytotoxicity (Schulze-Osthoff *et al.*, 1992). The increase in oxygen radical formation was followed by a marked decrease in mitochondrial

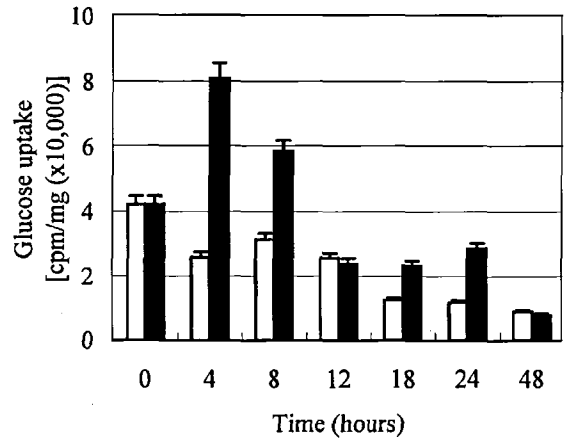


Fig. 6. Glucose uptake of L929 cells after TNF (50 units/ml) treatment for 4, 8, 12, 18, 24, and 48 h. Values are presented as mean ± SD of three independent experiments.

dehydrogenase activity and cellular ATP levels (Schulze-Osthoff *et al.*, 1992).

Besides mitochondrial degeneration, TNF-treated cells respond with an increased rate of glycolysis (Matthews *et al.*, 1987). The increased glycolytic activity accounts for enhanced glucose uptake and lactate production in the L6 muscle cell line, and similar results have been reported in synovial cells in culture (Talor *et al.*, 1988; Zentella *et al.*, 1993). The increase in glycolytic activity was also associated with a 10% decrease in cellular oxygen consumption and a comparable decrease in the production of CO_2. In this regard, it was previously reported that the principle driving force behind these changes might be the activation of a futile substrate cycle between 6-phosphofructo-1-kinase and fructose 1,6-bisphosphate phosphatase, resulting in the net hydrolysis of ATP and a consequent increase in glycolysis to compensate for the imposed energy loss (Zentella *et al.*, 1993).

In the present study, we compared the activities of glycolytic regulatory enzymes, lactate dehydrogenase, and pyruvate carboxylase between TNF-sensitive L929 cells and TNF-resistant A549 cells in order to define the parameters involved in distinguishing between cellular resistance and sensitivity to TNF cytotoxicity. The results indicate that all glycolytic regulatory enzymes activities increased markedly in TNF-treated L929 cells, whereas these enzyme activities did not increase significantly in TNF-treated A549 cells (Fig. 1). This suggests that glycolytic activity is not affected in TNF-resistant A549 cells after treatment with TNF. Since the lactate dehydrogenase activity, which is responsible for the continuous operation of glycolysis by supplying NADH, was also not affected upon TNF treatment in A549 cells (Fig. 2), the tricarboxylic acid cycle and oxygen consumption would normally proceed in this cell line. Furthermore, TNF treatment led to increased activity of

pyruvate carboxylase in TNF-resistant A549 cells but not in TNF-sensitive L929 cells (Fig. 3). This suggests that the high level of pyruvate carboxylase activity in TNF resistant A549 cells upon TNF-treatment accelerates the tricarboxylic acid cycle by conversion of pyruvate to oxaloacetate.

Previously, it was reported that TNF induces an increase in the intracellular levels of a potent allosteric regulator of 6-phosphofructo-1-kinase activity (Zentella *et al.*, 1993). Glucose uptake was also increased reaching a maximal level after 17 h in L6 myocytes upon TNF treatment (Zentella *et al.*, 1993). In order to understand the signal transduction pathway mediated by TNF, we measured the time-dependent intracellular cAMP concentration and glucose uptake in L929 cells after TNF treatment. As shown in Fig. 5, intracellular cAMP accumulation increased during 1–2 h and glucose uptake was maximal at 4 h after treatment with TNF. These data imply that the increase of cAMP and glucose concentrations resulted from the direct action of TNF, because the glycolytic enzyme activities were not affected significantly during these early hours in L929 cells upon TNF treatment. The increase of glycolytic enzymes activities were reduced to the level of the control when L929 cells were incubated with H-8, an inhibitor of cyclic nucleotide dependent protein kinase. Staurosporine and PMA, effectors of protein kinase C, did not affect the enzyme activities increased by TNF treatment. This suggests that increased glycolysis by TNF is mediated by the cAMP-dependent protein kinase A. It has been reported that protein kinase C down-regulates TNF receptors (O'Connell *et al.*, 1997), and arachidonate metabolism is related with TNF cytotoxicity (Suffys *et al.*, 1987). Since the increase of glycolysis was not affected either by protein kinase C effectors or arachidonic acid inhibitor in the present investigation, it can be concluded that TNF cytotoxicity is not directly related with the increase of glycolysis. Further study is necessary to reveal how the two TNF receptors (p55 and p75) mediate the activation of the cAMP-dependent protein kinase.

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Reference

Baik, N. G., Jeong, J. Y. and Kim, S. S. (1995) Size heterogeneity of murine tumor necrosis factors induced from mouse peritoneal macrophages. *J. Biochem. Mol. Biol.* (formerly *Korean Biochem J.*) **28**, 46–50.

Bergmeyer, H. U., Grabl, M. and Walter, H. E. (1983) in *Methods of Enzymatic Analysis*. Bergmeyrt, H. U. (ed.), vol. 2, pp. 222–223, Verlag Chemie, Weinheim, Germany.

Beutler, B. and Cerami, A. (1989) The biology of cachetin/TNF–α primary mediator of the host response. *Annu. Rev. Immunol.* **7**, 625–655.

Bradford, M. M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72**, 248.

Carswell, W. A., Old, L. J., Kassel, R. L., Green, S., Fiore, N. and Williamson, B. (1975) An endotoxin-induced serum factor that causes necrosis of tumors. *Proc. Natl. Acad. Sci. USA* **72**, 3666–3670.

Choeng, H. S. and Kim, S. S. (1994) Simple purification of recombinant human TNF-α by conventional chromatography. *J. Biochem. Mol. Biol.* (formerly *Korean Biochem J.*) **27**, 330–334.

Crane, J. K. and Sols, A. (1955) in *Methods in Enzymology*, vol. 1, pp. 277–286, Acadamic Press, New York, New York.

Dealtry, G. B., Naylor, M. S., Fiers, W. and Balkwill, F. R. (1987) DNA fragmentation and cytotoxicity caused by tumor necrosis factor is enhanced by interferon-gamma. *Eur. J. Immunol.* **17**, 689–693.

Evans, D. A., Jacobs, D. O. and Wilmore D. W. (1989) Tumor necrosis factor enhances glucose uptake by peripheral tissues. *Am. J. Physiol.* **257**, R1182–R1189.

Flick, D. A. and Gifford, G. E. (1984) Comparison of *in vitro* cell cytotoxic assays for tumor necrosis factor. *J. Immunol. Meth.* **68**, 167–175.

Fujii, H. and Miwa, S. (1983) in *Methods of Enzymatic Analysis*, Bergmeyrt, H. U. (ed.), vol. 3, pp. 496–501, Verlag Chemie, Weinheim, Germany.

Grunfeld, C. and Feingold, K. R. (1991) The metabolic effects of tumor necrosis factor and other cytokines. *Biotherapy* **3**, 143–158.

Hennet T., Richter, C. and Peterhans, E. (1992) Tumor necrosis factor-α induces superoxide anion generation in mitochondria of L929 cells. *Biochem. J.* **77**, 473–476.

Hori, T., Kashiyama, S., Hayakawa, M., Oku, N. and Ito, F. (1989) Tumor necrosis factor is cytotoxic to human fibroblasts in the presence of exogeneous arachidonic acid. *Exp. Cell Res.* **185**, 41–49.

Kim, S. S. and Kim, Y. H. (1997) Cellular resistance against tumor necrosis factor cytotoxicity in bcl-2 transfected L929 cells are mediated by manganous superoxide dismutase. *Cytokine* **9**, 247 (abstract).

Kim, Y. H. and Kim, S. S. (1999) Increase of MnSOD expression and decrease of JNK activity determine the TNF sensitivity in bcl2-transfected L929 cells. *Cytokine*, in press.

Lee, H., Kim, J. H. and Kim, S. S. (1989) Tumor necrosis factor from human peripheral monocytes: induction, purification, gene cloning, and expression. *J. Biochem. Mol. Biol.* (formerly *Korean Biochem J.*) **22**, 417–424.

Matthews N. (1983) Anti-tumour cytotoxin produced by human monocytes: Studies on its mode of action. *Br. J. Cancer* **48**, 405–410.

Matthews, N., Neale, M. L., Jackson, S. K. and Stark, J. M. (1987) Tumor cell killing by tumor necrosis factor: inhibition by anaerobic conditions, free-radical scavengers and inhibitors of arachidonate metabolism. *Immunol.* **62**, 153–155.

Mauduit, P., Zoukhri, D. and Rossignol, B. (1989) Direct activation of a protein kinase activity from rat lacrimal gland by PMA in a phopholipid-free system. *FEBS Lett.* **252**, 5–11.

McClure, W. R., Lardy, H. A. and Kneifel, H. P. (1971) Rat liver pyruvate carboxylase, 1. Preparation, properties, and cation specificity. *J. Biol. Chem.* **246**, 3569–3578.

Naichen, Y. U., Maciejewski-Lenoir, D., Bloom, F. E. and Magistretti, P. J. (1995) Tumor necrosis factor-α and interleukin-1α enhance glucose utilization by astrocytes: Involvement of phospholipase A_2. *Mol. Pharmacol.* **48**, 550–558.

O'Connell, M. A., Kelleher, D., Liskamp, R. M., Hall, N., O'Neill, L. A. and Long, A. (1997) TNF-mediated cytotoxicity of L929 cells: role of staurosphorine in enhancement of cytotoxicity and translocation of protein kinase C isozymes. *Cytokine* **9**, 83–92.

Polla, B. S., Jacquier-Sarlin, M. R., Kantengwa, S., Mariethoz, E., Hennet, T., Russo-Marie F. and Cossarizza, A. (1996) TNF-α alters mitochondrial membrane potential in L929 but not in TNF-α resistant L929.12 cells: relationship with the expression of stress proteins, annexin1 and superoxide dismutase activity. *Free Rad. Res.* **25**, 125–131.

Satomi, N., Sakurai, A., Haranaka, R. and Haranaka, K. (1988) Preventive effects of several chemicals against lethality of recombinant human tumor necrosis factor. *J. Biol. Response Mod.* **7**, 54–63.

Schulze-Osthoff, K., Bakker, A. C., Vanhaesebroeck, B., Beyaert, R., Jacob, W. A. and Fiers, W. (1992) Cytotoxic activity of tumor necrosis factor is mediated by early damage of mitochondrial functions. Evidence for the involvement of mitochondrial radical generation. *J. Biol. Chem.* **267**, 5317–5323.

Suffys, P., Beyoert, R., Van Roy, F. and Fiers, W. (1987) Reduced tumor necrosis factor-α induced cytotoxicity by inhibitors of the arachidonic acid metabolism. *Biochem. Biophys. Res. Commun.* **149**, 735–743.

Sugarman, B. J., Aggarwal, B. B., Hass, P. I., Figari, I. S., Palladino, M. A. and Shepard, H. M. (1985) Recombinant human tumor necrosis factor-α: effects on proliferation of normal and transformed cells *in vitro*. *Science* **230**, 943–945.

Takuma, T. (1988) Evidence against direct involvement of cyclic AMP-dependent protein phosphorylation in the exocytes of amylase. *Biochem. J.* **256**, 867–871.

Talor, D. J., Whitehead, R. J., Evanson, J. M., Westmacort, D., Feldmann, M., Bertfield, H., Morris, M. A. and Woolley, D. E. (1988) Effect of recombinant cytokines glycolysis and fructose-2,6-bisphosphate in rheumatoid synovial cells *in vitro*. *Biochem. J.* **250**, 111–115.

Vassault, A. (1983) in *Methods of Enzymatic Analysis*, Bergmeyrt, H. U. (ed.) vol. 3, pp. 118–126, Verlag Chemie, Weinheim, Germany.

Voelkel-Johnson, C., Entingh, A. J., Wold, W. S. M., Gooding, L. R. and Laster, S. M. (1995) Activation of intracellular proteases is an early event in TNF-induced apoptosis. *J. Immunol.* **154**, 1707–1716.

White, M. K., Bramwell, M. E. and Harris, H. (1981) Hexose transport in hybrids between malignant and normal cells. *Nature* **294**, 232–235.

Wong, G. H. W., Elwell, J. H., Oberly, L. W. and Goeddel, D. V. (1989) Manganous superoxide dismutase is essential for cellular resistance to cytotoxicity of tumor necrosis factor. *Cell* **58**, 923–931.

Zentella, A., Manogue, K. and Cerami, A. (1993) Cachetin/TNF-mediated lactate production in cultured myocytes is linked to activation of a futile substrate cycle. *Cytokine* **5**, 436–447.

Zimmerman, R. J., Chan, A. and Leadon, S. A. (1989) Oxidative damage in murine tumor cells treated *in vitro* by recombinant human tumor necrosis factor. *Cancer Res.* **49**, 1644–1648.