Retinoic acid inhibits inducible nitric oxide synthase expression in 3T3-L1 adipocytes

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Abbreviations: IFN- γ , interferon- γ ; iNOS, inducible nitric oxide synthase; LPL, lipoprotein lipase; LPS, lipopolysaccharide; RA, retinoic acid; RAR, retinoic acid receptor; RXR, retinoid-X receptor; TNF, tumor necrosis factor- α .

Abstract

The present study was undertaken to explore whether retinoids, which are known to have immunomodulatory actions, could attenuate tumor necrosis factor- α (TNF)-stimulated inducible nitric oxide synthase (iNOS) expression in 3T3-L1 adipocytes. Adipocytes incubated with TNF induced dose- and time-dependent accumulation of nitrite in the culture medium through the iNOS induction as confirmed by Western blotting. Treatment of cells with TNF in the presence of all-*trans*-retinoic acid (RA) significantly decreased their ability to produce nitrite and iNOS induction. Both 13-*cis*- and all-*trans*-RA-induced suppression was dose-dependent, and all-*trans*-RA was somewhat potent than 13-*cis*-RA. The inhibitory effect of RA on TNF-induced iNOS induction was reversible, completely recovered after 2 days, and was exerted through the inhibition of NF- κ B activation. TNF also suppressed the lipoprotein lipase (LPL) activity of 3T3-L1 adipocytes. RA could not reverse the TNF-induced LPL suppression at RA levels causing near complete inhibition of the TNF-induced NO production. These results indicate that RAs attenuate iNOS expression reversibly in TNF-stimulated 3T3-L1 adipocytes, and that the TNF-

induced LPL suppression is not the result of NO overproduction.

Keywords: adipocytes, retinoic acid receptors, nitric oxide, nitric oxide synthase, tretinoin, tumor necrosis factor

Introduction

Adipose tissue is widely distributed in the body, representing as much as 15 to 25% of the total body weight in normal adults (Gray, 1989). Anatomically, adipose tissue is closely related with immune system. In mammals, nearly all lymph nodes always occur embedded in and firmly attached to adipose tissue. The adipose tissue around the lymph nodes is the last depot (apart from bone marrow and the structural and cardiac depots) to be depleted in very lean wild mammals (Pond, 1996). Thymus is also closely associated with adipose tissues and is gradually replaced by it.

Acute systemic immune responses are almost invariably accompanied by anorexia and major changes in whole body lipid metabolism (Grunfeld and Feingold, 1992). The fatty acids released into the circulation are believed to be important to immune systems as an energy source, as components of membrane, and as substrates for the synthesis of complex lipids such as leukotrienes, prostacyclins, thromboxane, and prostaglandins (Pond, 1996).

Adipocyte itself secretes and responds vigorously to various cytokines secreted by immune cells. For example, adipose tissue appears to be an important source of tumor necrosis factor- α (TNF) and is also a target for TNF (Fruhbeck *et al.*, 2001). Moreover, TNF has been shown to be involved in obesity-linked insulin resistance (Hotamisligil *et al.*, 1993). The action of TNF seems to depend upon its source within body as well as its overall concentration in venous blood, strongly implicating the local interactions between adjacent tissues (Tracey and Cerami, 1992).

Recently, there are reports that adipose tissue is a potential site of nitric oxide (NO) production in endotoxemia (Ribiere *et al.*, 1996; Kapur *et al.*, 1999). After endotoxin challenge, NO production in adipose tissues increased markedly and to levels comparable to that of the liver and greater than that of muscle and kidney (Kapur *et al.*, 1999). Considering the mass and diffuse distribution of adipose tissue, adipose

tissue could significantly contribute to the overall local NO production.

NO is an important messenger molecule that plays a critical role in a variety of physiological functions, including neuronal transmission, vascular relaxation, immune modulation, and cytotoxicity (Moncada *et al.*, 1991). Three isotypes of NO synthase (NOS) were known: the calcium-dependent endothelial NOS, the neuronal type NOS and the calcium-independent inducible NOS (iNOS). Among those isotypes, iNOS can produce large amount of NO over the prolonged periods (Xie and Nathan, 1994). iNOS induction by endotoxin causes an enhanced formation of NO that contributes to hypotension, vascular hyporeactivity to vascular constrictors, organ injury, and dysfunction (Moncada *et al.*, 1991; Xie and Nathan, 1994). Negative regulators of iNOS may therefore be able to attenuate NO-mediated cytotoxicity.

Retinoids are a group of natural or synthetic derivatives of vitamin A that exert anti-inflammatory and immunomodulatory actions (Stephensen, 2001). Isomers of retinoic acid (RA), the main active metabolite of vitamin A, activate retinoid receptors that act as ligand-dependent transcription factors and affect gene expression (Mangelsdorf *et al.*, 1995; Villarroya *et al.*, 1999).

Adipose tissues are major sites of storage of vitamin A derivatives. It has been estimated that all adipose depots could account for 15-20% of the total body retinoid stores (Tsutsumi *et al.*, 1992). They play an active role in whole body metabolism of retinoids (Tsutsumi *et al.*, 1992). Moreover, adipose tissues are targets for the action of retinoic acid, and retinoid receptors belonging to the two different subfamilies, RA receptors (RARs) and the retinoid-X receptors (RXRs), are expressed in adipose tissues (Villarroya *et al.*, 1999).

As indicated by previous research, the ability of RA to modulate NO production may differ within various contexts of infection or inflammation. Some studies have demonstrated the potential of RA to reduced iNOS gene expression or activity (Hirokawa *et al.*, 1994; Mehta *et al.*, 1994; Becherel *et al.*, 1996; Motomura *et al.*, 1997; Datta and Lianos, 1999), implying an anti-inflammatory effect of RA treatment, while others have demonstrated RA's potential to increase NO production (Dugas *et al.*, 1996; Ghigo *et al.*, 1998; Norford *et al.*, 1998; Devaux *et al.*, 2000; Martin *et al.*, 2000; Austenaa and Ross, 2001), suggesting a proinflammatory action. An understanding of how retinoid exposure affects cellular responses to immune stimuli is of both basic and practical importance.

In this study using 3T3-L1 adipocytes, we observed that large amount of NO was produced by TNF through the iNOS induction, and that RA inhibited the

TNF-induced NO production of adipocytes through the inhibition of NF- κ B activation

Materials and Methods

Culture of 3T3-L1 adipocytes

3T3-L1 cells were grown to confluence with standard medium (DMEM containing 10% fetal bovine serum, 100 U/ml penicillin, 0.1 mg/ml streptomycin, and 0.25 μ g/ml amphotericin B) in 75 cm² culture flask as described by Park *et al.* (1997). Confluent cells were stimulated to differentiate into adipocytes by supplementing standard medium with 1 μ M dexamethasone (Sigma, St. Louis, MO), 10 μ g/ml insulin (Sigma), and 0.5 mM isobutylmethylxanthine (Sigma) for 2 days. The medium was then replaced with standard medium containing 5 μ g/ml insulin and was changed every 2 days. The cultured cells were used for experiments within 5-6 days after confluence.

Peritoneal exudate macrophages were obtained from ICR mice injected with 2 ml of sterile 3% thioglycolate (Difco) as described previously (Kang *et al.*, 2002). Obtained macrophages (4×10^5 cells) were cultured with RPMI 1640 medium supplemented with 10% fetal bovine serum, 100 U/ml penicillin G, and 5 μ g/ml streptomycin (total volume of 400 μ l) in 24-well culture plate with lipopolysaccharide (LPS) from *Escherichia coli* serotype 0127:B8 (50 ng/ml, Sigma), recombinant mouse interferon- γ (IFN- γ , 5 U/ml, Sigma) and TNF (500 U/ml, Sigma) for 48 h.

Nitrite measurement

Biologically produced NO is rapidly oxidized to nitrite and nitrate in aqueous solutions (Moncada *et al.*, 1991). Nitrite concentration in the cell-free culture supernatant, therefore, served as a reflection of NO production and was measured using a colorimetric assay (Green *et al.*, 1982). Following a 48 h incubation in 24-well plates at 37°C in a humidified 95% air/5% CO₂ atmosphere, nitrite concentration was measured in the cell free culture supernatant. Briefly, 50 μ l aliquots of the culture supernatants dispensed into 96-well microtiter plates (flat bottom) were incubated with 100 μ l of a 1:1 mixture of 1% sulfanilamide (Sigma) in 30% acetic acid and 0.1% *N*-(1-naphthyl) ethylenediamine dihydrochloride (Sigma) in 60% acetic acid at room temperature. After 5 min, absorbance was measured at 540 nm using a microtiter plate reader (MR 700, Dynatech Laboratories). Concentrations were determined from a linear standard curve obtained from serial dilution of sodium nitrite (Sigma) in working medium.

Western blot for iNOS

Cells were homogenized in 100 μ l of ice cold lysis buffer (20 mM HEPES, pH 7.2, 1% Triton X-100, 10% glycerol, 1 mM phenylmethylsulfonyl fluoride, 10 μ g/ml leupeptin, 10 μ g/ml aprotinin). The homogenates containing 20 μ g of protein were separated by SDS-PAGE with 10% resolving and 3% acrylamide stacking gel (Laemmli, 1970), and transferred to nitrocellulose sheets (Schleicher and Schuell, Dassel, Germany) in a Western blot apparatus (Bio-Rad) run at 50 V for 2 h.

The nitrocellulose paper was blocked with 2% bovine serum albumin and then incubated for 4 hr with 1 μ g/ml anti-mouse macrophage iNOS rabbit IgG (Transduction Laboratories). The binding of antibody was detected with anti-rabbit IgG conjugated to an alkaline phosphatase (Sigma). Immunoblots were developed using a BCIP/NBT solution (Pierce).

Electrophoretic mobility shift assay

The nuclear extracts were prepared from the TNF-treated adipocytes according to the method of Im *et al.* (1997). To inhibit endogenous protease activity, 1 mM phenylmethylsulfonyl fluoride was added. Protein contents in nuclear extracts were measured using the Bradford method (Bradford, 1976).

Electrophoretic mobility shift assay was performed as described previously (Park and Park, 2001). As a probe for the gel retardation assay, NF- κ B specific oligonucleotide (κ B, 5'-CCG GTT AAC AGA GGG GGC TTT CCG AG-3') was synthesized. The two complementary strands were annealed and labeled with [α - ^{32} P]dCTP. Labeled oligonucleotides (10,000 cpm), 10 μ g of nuclear extracts, and binding buffer (10 mM Tris-HCl, 500 mM KCl, 10 mM EDTA, 50% glycerol, 100 ng of poly (dI·dC), and 1 mM DTT, pH 7.6) were incubated for 30 min at room temperature in a final volume of 20 μ l. The reaction mixture was analyzed by electrophoresis on a 4% polyacrylamide gel in 0.5 $\times$ Tris-borate buffer. Specific binding was controlled by competition with a 50-fold excess of cold κ B oligonucleotide.

Assay of lipoprotein lipase (LPL) activity

At the end of experiment, cells were rinsed with ice-cold phosphate-buffered saline, harvested with 0.5 ml of MNT-buffer [20 mM 2-(*N*-morpholino)ethanesulfonic acid, 100 mM NaCl, 30 mM Tris, and 1 mM CaCl₂, pH 7.6] containing 1% Triton X-100 (Park *et al.*, 1997), and sonicated briefly at 4°C. The suspension was centrifuged at 15,000 $\times g$ for 20 min at 4°C, and the infranant was used for LPL activity assay.

A stock triacylglycerol emulsion containing 5 mCi of tri[9,10 (n- ^{3} H)] oleoylglycerol (Amersham), 1.13

mmol of trioleoylglycerol (Sigma), 60 mg of 1- α -phosphatidylcholine (bovine liver, Sigma) and 9 ml of glycerol was prepared according to the method of Nilsson-Ehle and Schotz (1976). Before assay, 1 vol. of stock emulsion, 19 vol. of 3% bovine serum albumin in 0.2 M Tris-HCl buffer (pH 8.1) and 5 vol. of heat-inactivated fasted rat serum (heated at 60°C for 30 min) were mixed and incubated for 30 min. For assay, 100 μ l of this activated substrate mixture was added to a same amount of enzyme solution, and incubated at 37°C for 60 min. Released fatty acids were extracted and measured as described previously (Park *et al.*, 1997). One mU of LPL activity represents the release of 1 nmol fatty acid/min.

Results

NO production from 3T3-L1 adipocytes

Incubation of 3T3-L1 adipocytes with TNF (50 ng/ml) led to the accumulation of nitrites in the culture medium (Figure 1). Nitrite accumulation was slow during first 24 h, then, continued to increase linearly up to 4 days. TNF-induced nitrite production was also dose-dependent, with 50% of maximal nitrite accumulation occurring at a TNF dose of about 28 ng/ml (Figure 2). Maximal accumulation was observed over the TNF concentration of 50 ng/ml. These results indicate that nitrite accumulation in the culture medium can be induced by TNF, most likely as a consequence of increased NOS activity, in 3T3-L1 adipocytes. TNF-induced nitrite production was not observed in undifferentiated, fibroblast-like 3T3-L1 cells (data not shown).

Lysate of 3T3-L1 adipocytes treated with TNF was

Figure 1. Time-dependent nitrite production by TNF in 3T3-L1 adipocytes. 3T3-L1 adipocytes were incubated with 50 ng/ml TNF. Nitrites in the medium was measured using the Greiss reagent. Each point represents the mean $\pm$ S.E.M. of three independent experiments.

Figure 2. Concentration-dependent nitrite production by TNF in 3T3-L1 adipocytes. 3T3-L1 adipocytes were incubated with various concentrations of TNF for 48 h. Nitrites in the medium was measured using the Greiss reagent. Each point represents the mean $\pm$ S.E.M. of three independent experiments.

Figure 3. Effect of TNF and RA on the expression of iNOS in 3T3-L1 adipocytes. 3T3-L1 adipocytes (lane 1-4) were incubated with 50 ng/ml TNF and/or 100 μ M all-*trans*-RA for 48 h. Mouse peritoneal macrophage (lane 5-6) was incubated with 50 ng/ml *E. coli* LPS and 5 U/ml interferon- γ for 48 h. Cell lysate was separated on SDS-PAGE and transferred to nitrocellulose membrane. iNOS protein was visualized with antibody for mouse macrophage iNOS. The arrow indicates the 130 kDa iNOS protein. A representative experiment was reported. Lane 1-4, adipocytes; lane 1, none; lane 2, TNF; lane 3, RA; lane 4, TNF and RA. Lane 5-6, macrophages; lane 5, none; lane 6, LPS and IFN- γ .

subjected to immunoblot analysis using an antibody prepared against the mouse macrophage iNOS (Figure 3). A major protein band of 130 kDa was detected in adipocytes treated with 50 ng/ml TNF for 48 h (lane 2). Mouse peritoneal macrophages treated with 50 ng/ml *E. coli* LPS and 5 U/ml interferon- γ were used as a positive control (lane 6). iNOS band was not detected in unstimulated adipocytes (lane 1) and macrophages (lane 5). These results indicate that an iNOS with the same molecular and antigenic properties with murine macrophage iNOS is induced by TNF in 3T3-L1 adipocytes.

Inhibition of TNF-induced NO production by RA

Unstimulated adipocytes produced nearly undetectable amounts of nitrites in the presence and absence of RA (100 μ M, 48 h), whereas stimulation of cells

Figure 4. Effect of RA on the TNF-induced production of nitrite production. 3T3-L1 adipocytes were treated with 50 ng/ml TNF in the presence or absence of all-*trans*-RA (100 μ M) for 48 h, and nitrite in the medium was measured using the Greiss reagent. Each point represents the mean $\pm$ S.E.M. of three independent experiments.

Figure 5. Concentration-dependent inhibition of all-*trans*- and 13-*cis*-RA on the TNF-induced nitrite accumulation from 3T3-L1 adipocytes. 3T3-L1 adipocytes were incubated with 50 ng/ml TNF in the presence of various concentrations of all-*trans*- and 13-*cis*-RA for 48 h. Nitrites in the medium was measured using the Greiss reagent. Each point represents the mean $\pm$ S.E.M. of three independent experiments.

with TNF (50 ng/ml) induced high levels of nitrite (Figure 4). Treatment of cells with TNF in the presence of 100 μ M all-*trans*-RA significantly decreased their ability to produce nitrite in response to TNF. The iNOS protein of 130 kDa in Western blot induced by TNF was also abolished by the co-incubation with RA (Figure 3, lanes 3 and 4).

Two isomers of RA, all-*trans* RA and 13-*cis* RA exhibited the similar suppressive effects on TNF-induced nitrite production (Figure 5). Both RAs-mediated suppression of nitrite production was concentration-dependent, reaching to the maximal inhibition at 100 μ M, while all-*trans*-RA is somewhat potent than 13-*cis*-RA.

Figure 6. Effect of TNF and RA withdrawal on the TNF-induced nitrite production by 3T3-L1 adipocytes. 3T3-L1 adipocytes were treated with TNF (50 ng/ml) in the presence or absence of all-*trans*-RA (100 μM) for 48 h. After extensive washing, cells were incubated for 0-2 days in the culture medium alone. Then the same condition of TNF and RA were maintained for another 48 h, and nitrite in the medium was determined. Treatment conditions were expressed as 'pretreatment/treatment after washing' in the figure. Each point represents the mean $\pm$ S.E.M. of three independent experiments.

To demonstrate that RA and TNF used in these experiments were not toxic to adipocytes, we investigated whether treated cells could recover their nitrite production capacity. Cells were treated with TNF (50 ng/ml) in the presence or absence of all-*trans*-RA (100 μM) for 48 h. After extensive washing, cells were incubated for 0-2 days in the culture medium alone. Then the same condition of TNF and RA were maintained for another 48 h and the nitrite accumulated in the medium was determined. As shown in Figure 6, the suppressive effect of RA was completely recovered 2 days after washing out. These results indicate that used concentrations of TNF and RA are not cytotoxic and that the inhibitory effect of RA is reversible.

Because NF-κB is implicated in the transcriptional regulation of cytokine-induced iNOS expression, the effect of RA on TNF-stimulated translocation of NF-κB from cytosol to nucleus was investigated (Figure 7). The nuclear extract from TNF-stimulated adipocytes showed an great increase in NF-κB binding activity (lane 2) comparing with those of unstimulated (lane 1) and RA treated cells (lane 3). The TNF-stimulated binding activity was markedly suppressed by the addition of RA (lane 4), implying that RA inhibits iNOS expression and NO production through the inhibition of NF-κB activation. Specificity of the DNA-protein interaction for NF-κB was demonstrated by a competitive assay with a 50-fold excess of unlabeled oligonucleotides (lane 5).

Figure 7. Effect of RA on the TNF-induced activation of NF-κB in 3T3-L1 adipocytes. 3T3-L1 adipocytes were incubated with 50 ng/ml TNF and/or 100 μM all-*trans*-RA for 30 min. Nuclear extracts of cells were incubated with ^{32} P-labeled κB oligonucleotides and electrophoresed on a 4% polyacrylamide gel. A 50-fold excess of cold κB was added as competitors. A representative experiment is reported. Lane 1, none; lane 2, TNF; lane 3, RA; lane 4, TNF and RA; lane 5, 50-fold excess of cold κB.

Figure 8. Effect of RA on the TNF-induced reduction of LPL activity and increase of nitrite production. 3T3-L1 adipocytes were treated with 50 ng/ml TNF in the presence or absence of all-*trans*-RA (100 μM) for 48 h. LPL activity from cell lysate and nitrites in the medium was determined as described in the Materials and Methods. Each point represents the mean $\pm$ S.E.M. of three independent experiments.

NO and LPL activity

TNF is also known as cachectin, which was first discovered because it decreased adipose tissue LPL activity (Beutler and Cerami, 1988). Recently, there were reports that NO mediate the down regulation of LPL activity induced by TNF in adipocytes (Uchida *et al.*, 1997a; 1997b). As RA could suppress the TNF-induced NO production, we investigated the effect of RA on TNF-induced down-regulation of LPL activity.

When cells were treated with 50 ng/ml TNF for 48 h, NO production was markedly increased and LPL activity showed about 60% decrease (Figure 8). Addition of RA (100 μM) in the presence of TNF caused nearly complete suppression of NO production. However, LPL activity was not recovered, rather decreased to 17% of control cells. RA alone caused about 27% decrease of cellular LPL activity. These results indicate that increased NO production by TNF

does not mediate the TNF-induced down-regulation of LPL activity in 3T3-L1 adipocytes.

Discussion

The treatment of adipocytes with TNF caused significant increase of NO production through the induction of iNOS. The synergistic effect of IFN with low dose of TNF on NO production was also observed in adipocytes as in macrophages. However, the IFN doses needed to invoke similar extent of synergistic effect were much higher in adipocytes (data not shown). TNF-induced NO production was observed only in fully differentiated adipocytes, not in undifferentiated, fibroblast-like preadipocyte cells.

The physiologic significance of iNOS induction in adipose tissue is so far unknown. However, considering the mass and its diffuse distribution, adipose tissue could significantly contribute to the overall local NO production. This suggests that adipocytes may play a role in the local immune defense during the inflammatory processes.

In addition to the close anatomical association with immune systems including lymph nodes and thymus, adipose tissue is also known as a major site of expression of immunoregulatory molecules. TNF is produced from adipose tissues (Hotamisligil *et al.*, 1993) and acts on it (Fruhbeck *et al.*, 2001). Adipocyte-specific proteins, such as adiponin or leptin, also play a role in immune reactions. Adiponin is a murine equivalent of human complement factor D that is the initial and rate-limiting enzyme of the alternative pathway of complement activation (Rosen *et al.*, 1989). Leptin also enhances cytokine production and phagocytosis by macrophages (Gainsford *et al.*, 1996). Thus, adipose tissue seems to represent an important participant of immune reactions, and the concerted actions of TNF, iNOS expression and other cytokines strongly support a role of this tissue in immune function.

The liver is the main organ involved in the storage and homeostasis of retinoids. However, adipose tissue contains 15-20% of the total body retinol and retinyl esters (Tsutsumi *et al.*, 1992). Retinoids accumulate in adipocytes and the differentiation of precursor cells into adipocytes is associated with accumulation of intracellular retinoids (Villarroya *et al.*, 1999). Adipose tissue retinoid stores may be derived through the LPL hydrolysis of the retinyl esters contained in postprandial chylomicrons, or through the uptake from blood stream by retinol-binding protein. Moreover, adipocytes synthesize retinol-binding protein *in vitro* and secrete into the culture medium, and retinol can be mobilized from adipocytes (Tsutsumi *et al.*, 1992; Villarroya *et al.*, 1999). Thus, adipose tis-

sues regulate retinoid homeostasis, as they are involved not only in retinol uptake and storage, but also in its mobilization and transport.

The biological effects of RA isomers involve the activation of ligand-dependent transcription factors, the retinoid receptors. These belong to the nuclear receptor superfamily, which includes the steroid, vitamin D and T₃ receptors, the peroxisome proliferator-activated receptors and a number of orphan receptors whose ligands are unknown. Adipose tissues have long been recognized as a potential site for RA action as both RARs and RXRs are expressed in adipose tissues (Villarroya *et al.*, 1999). All-*trans*-RA influences gene expression by activating the RAR moieties of RAR/RXR heterodimers that bind RA response elements (Mangelsdorf *et al.*, 1995).

However, little is known about their action on adipocytes during inflammation. Previous reports about the relationship between RA and NO were somewhat controversial within various contexts of infection or inflammation, from anti-inflammatory to pro-inflammatory actions. RA inhibits NOS induction in mesangial cells (Datta and Lianos, 1999), Kupffer cell (Motomura *et al.*, 1997), keratinocytes (Becherel *et al.*, 1996), peritoneal macrophages (Mehta *et al.*, 1994) and vascular smooth muscle (Hirokawa *et al.*, 1994). However, RA-induced enhancement of NO synthesis has been observed *in vivo* (Devaux *et al.*, 2000), and in several cell types including monocytic cells (Dugas *et al.*, 1996), breast cancer cells (Martin *et al.*, 2000), epithelial cells (Norford *et al.*, 1998), neuroblastoma cell line (Ghigo *et al.*, 1998) and in RAW 264.7 cells (Austenaa and Ross, 2001). Our study demonstrated that RA could down-regulate the iNOS induction dose-dependently and reversibly in TNF-treated adipocytes and that the suppression of iNOS induction occurred through the inhibition of NF- κ B activation. However, relatively high dose of RA (10-100 μ M) was needed in adipocytes for the down-regulation of iNOS induction. The requirement of high RA in adipocytes might be due to the lowering of effective drug concentration by the solubilization of RA into the relatively inert lipid droplets.

TNF is well documented to play an important role in wasting syndrome (cachexia) in infection or malignancy, which is associated with diverse abnormalities in carbohydrate, protein, and lipid metabolism (Beutler and Cerami, 1988). Recently, there were reports that NO mediate the down regulation of LPL activity induced by TNF in adipocytes (Uchida *et al.*, 1997a; 1997b). As RA could suppress the TNF-induced NO production, the effect of RA on TNF-induced down-regulation of LPL activity was investigated. In our study, inverse relationship between LPL activity and accumulation of nitrite was observed after the addition of TNF. However, In the presence of RA, TNF-in-

duced NO production was completely suppressed while the down-regulation of LPL activity was not reversed. These results indicate that increased NO production by TNF does not mediate the TNF-induced down-regulation of LPL activity in 3T3-L1 adipocytes, as reported by Picard *et al.* (2001).

NO is a double-edged sword in that it is critical for host survival in some conditions but also detrimental to the host in other inflammatory conditions. The marked NO production consequent to iNOS expression in endotoxic shock is believed to cause hypotension, organ injury, and dysfunction (Moncada *et al.*, 1991; Xie and Nathan, 1994). Therefore, our results support RA as a naturally occurring, nontoxic means to attenuate iNOS expression and NO-mediated cytotoxicity in adipocytes.

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