Review

Liposomes as Immunomodulator —Inhibitory Effect of Liposomes on NO Production from Macrophages—

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Liposomes have been artificially made into membranous vesicles composed essentially of naturally occurring phospholipids and have been found to serve as a carrier of drugs and an immunological adjuvant. After being intravenously injected, they are quickly removed from the blood circulation and trapped by Kupffer cells of the liver and macrophages of spleen. However, the changes liposomes exert in these cells with which liposomes interact remain unresolved. To clarify this point is very important to assure the safe use of liposomes as drug carriers. Macrophages have many unique functions, and nitric oxide (NO) produced by NO synthase (NOS) which is induced in response to some cytokines and bacterial products such as lipopolysaccharide (LPS) is responsible for the bactericidal, tumoricidal and immune regulatory activities. On the other hand, overexpressed NO is implicated in the development of atherosclerosis, DNA injury, and hypotension associated with septic shock. This article focuses on the effects of liposomes on NO production from LPS-stimulated mice peritoneal macrophages *in vitro*; we found that liposomes composed of phosphatidylserine inhibit NO production. We also discuss the mechanism of the inhibitory activity of liposomes.

Key words macrophage; liposome; nitric oxide

INTRODUCTION

Liposomes are of considerable interest as carriers for the controlled delivery of drugs because many substances can be encapsulated in aqueous and lipid phases.¹⁾ Cationic liposomes are particularly expected to be a candidate as a non-viral vector, because DNA readily forms a complex with cationic liposomes *via* electrostatic interactions^{2–4)}; liposomes labeled with the ligands transferrin,⁵⁾ folate,⁶⁾ epidermal growth factor (EGF),⁷⁾ and asialoglycoprotein^{8,9)} are reported to be candidate carriers of DNA to the expected tissues *in vivo*.

One of the best known observations in the field of liposome research is that intravenously injected naked liposomes are rapidly taken up by macrophages in the reticuloendothelial system such as the liver and spleen.^{10,11)} Since this characteristic is advantageous for directing antigens or immunomodulating agents toward macrophages, many investigators have used liposomes as an adjuvant, depots for the slow release of antigens, and the targeting antigens to macrophages, which are one of the antigen-presenting cells. Macrophages have many unique functions, such as enhancing phagocytosis, protease secretion, increasing the production of interleukin-1, and cellular cytotoxicity, when activated by various naturally occurring synthetic agents.¹²⁾ But, it is not yet clear how liposomes affect these macrophage

functions. It is essential that this be clarified to assure that liposomes can be used safely as controlled delivery systems. We previously investigated the effects of liposomes on Fcγ-receptor-mediated phagocytosis, one of the most important functions of macrophages, *in vitro*¹³⁾ and *in vivo*,^{14,15)} and found that they activated macrophages to phagocytize the opsonized foreign body through Fcγ-receptor irrespective of liposomal charge; this suggested that liposomes could alter macrophage functions.

Nitric oxide (NO), a reactive free-radical gas, was found to be generated enzymatically from L-arginine and molecular oxygen by constitutive or inducible NO synthase (NOS) in a variety of cells; neuronal cells, endothelial cells, neutrophils, Kupffer cells, and activated macrophages (Table 1).^{16,17)} Endothelial and neuronal NOS are expressed constitutively and are Ca²⁺ and calmodulin dependent, and NO produced by these NOS acts as an intracellular messenger, participating in neurotransmission and vascular homeostasis.^{18,19)} In macrophages, NOS is expressed only by transcriptional induction, and macrophages activated with some cytokines or lipopolysaccharide (LPS) produce large quantities of NO which is generated by inducible NOS (i-NOS). NO produced by i-NOS in macrophages is partially responsible for the bactericidal, tumoricidal, and possibly immune regulatory activities. Once induced, i-NOS synthesizes high levels of NO at a constant rate for long periods, and overex-

Table 1. Characteristics of Three Isoforms of NOS

	Representative cell	Expression	Regulation of NO production
n-NOS (NOS I)	Neuron	Constitutive	Ca ²⁺ /calmodulin dependent
e-NOS (NOS III)	epithelial cell Endothelial cell	Constitutive	Ca ²⁺ /calmodulin dependent
i-NOS (NOS II)	Macrophage hepatocyte	Inducible	Depends on amount of i-NOS protein but not on Ca ²⁺ /calmodulin

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pressed NO and its metabolite ONOO^- have been implicated in the development of atherosclerosis,²⁰ DNA injury,²¹ and hypotension associated with septic shock.^{22–24} Consequently, it is crucial to regulate the excess NO produced by i-NOS in macrophages.

This article describes the inhibitory effects of liposomes on the NO production from mouse peritoneal macrophages stimulated with LPS *in vitro* and the inhibitory mechanism of action.

Effects of Liposomes on NO Production from Macrophages C3H/HeN mice (male, 6–8 weeks old) were injected with 1 ml of 2% thioglycollate intraperitoneally. On day 4, peritoneal macrophages were collected and then incubated with liposomes for 24 h. The macrophages were then further incubated with 10 $\mu\text{g}/\text{ml}$ LPS for 48 h in order to elicit NO production. NO production was estimated by measurement of nitrite in culture supernatant using Griess reagent.²⁵ As shown in Fig. 1(A), NO production induced by LPS was inhibited by the treatment of macrophages with negatively charged liposomes composed of phosphatidylserine (PS-liposomes, phosphatidylcholine (PC):PS:cholesterol=1:2:1 (by mol)) and phosphatidic acid (PA-liposomes, PC:PA:cholesterol=1:2:1), while no inhibition was observed in neutral liposomes composed of phosphatidylcholine (PC-liposomes, PC:cholesterol=3:1). PS-liposomes inhibited NO production in a dose dependent manner, and complete inhibition was observed at 500 $\mu\text{g}/\text{ml}$ of PS (Fig. 1(B)).²⁶ No differences in the inhibitory activities of PS-liposomes were observed between multilamellar vesicles and small unilamellar vesicles. Furthermore, macrophage viability after liposome treatment was estimated by Trypan blue dye exclusion test, and no change in viability was observed (data not shown). Hence, NO production was inhibited by negatively charged liposomes.

The kinetics of NO inhibitory activity of PS-liposomes on macrophages was examined, and at least 21 h of PS-liposome treatment were needed to decrease NO production to control levels (Fig. 2). Preincubation of macrophages with PS-liposomes for 3, 6, or 9 h resulted in no inhibition of their potential to produce NO; nor did the addition of PS-liposomes to macrophage culture at the time of LPS stimulation interfere with their normal production of NO. Therefore, pretreatment appears to be required in order to observe the inhibitory activity of PS-liposomes in NO production induced by LPS.²⁶

Figure 3 shows the results of Western blot analysis for i-NOS protein. An immunoreactive band at 130 kDa reacting with anti-i-NOS antibody was observed in the extract of the control and PC-liposome-treated macrophages, whereas a faint or no band was observed in PS-liposomes-treated ones. The intensity of the immunoreactive band was inversely pro-

portional to the PS concentrations, and the band was completely diminished when macrophages were treated with 500 $\mu\text{g}/\text{ml}$ of PS-liposomes. These results indicate that PS-li-

Fig. 1. Effects of Various Liposomes (A) and Liposomal Dose (B) on NO Production from Macrophages Stimulated with LPS

In A, peritoneal macrophages (1×10^5 cells/ml) were incubated for 24 h with PC-, PA-, and PS-liposomes (500 μg phospholipid/ml), and further incubated for 48 h with or without LPS (10 $\mu\text{g}/\text{ml}$). The supernatant of macrophage cultures was collected and NO production was evaluated by measuring nitrite. In B, peritoneal macrophages were incubated with indicated dose of PC- (■), and PS-liposomes (●) for 24 h, and then incubated for 48 h in the presence of LPS (10 $\mu\text{g}/\text{ml}$). Macrophages were treated with buffer (○) instead of LPS. The values are the mean $\pm$ S.D. of triplicate cultures from three independent experiments.

Fig. 2. Effects of Time of PS-Liposome Addition on the LPS Activation of Macrophages to Produce Nitrite

PS-liposomes (500 μg phospholipid/ml) were added to the macrophage culture at the indicated time before activation with LPS (10 $\mu\text{g}/\text{ml}$), and nitrite was measured 48 h later.

Fig. 3. Immunoblotting Analysis of Mouse Peritoneal Macrophages by the Antiserum Raised against i-NOS

(A) Macrophages were treated with buffer (HBSS), PC-liposomes, or PS-liposomes for 24 h, and further incubated with LPS (10 $\mu\text{g}/\text{ml}$) for 48 h. Macrophage lysates were subjected to Western blotting. (B) The effects of PS-liposomal dose on the induction of i-NOS was investigated.

Fig. 4. Effects of Liposomes on the Expression of i-NOS mRNA

Macrophages (1×10^6 cells) were treated with 500 μ g phospholipid/ml of PC-liposomes (A) or PS-liposomes (B) for the indicated times. Total RNAs were extracted and then RT-PCR was performed. Lower panel shows the changes in ratio of i-NOS mRNA to β -actin which was evaluated from the band intensity using NIH image.

Table 2. Scavenger Receptor and Ligand Binding

	SRA		SRB		SRC	Fc γ RIIB2	Macrosialin
	AI & AII	MARCO	CD36	SR-BI	SR-CI		
OxLDL	+	N.D.	+	+	N.D.	+	+
AcLDL	+	+	-/+	+	+	-	-/+
Maleylated BSA	+	N.D.	+	+	+	N.D.	N.D.
Dextran sulfate	+	N.D.	-	-	+	N.D.	N.D.
PS-liposome	-	N.D.	+	+	N.D.	N.D.	+

N.D., not determined. Fukasawa, *Seikagaku*, **68** (5), 373 (1996).

posomes inhibit the i-NOS expression in macrophages following LPS stimulation, but do not inhibit i-NOS activity.²⁶⁾ The effects of liposomes on i-NOS mRNA levels were also examined by RT-PCR analysis. Figure 4(A) indicates the effects of PC-liposomes on i-NOS mRNA levels following LPS stimulation. The i-NOS mRNA band was observed at 3 h, and reached the maximum level at 6 h after stimulation with LPS. The changes in ratio of i-NOS mRNA to β -actin mRNA were estimated by NIH image, and no changes in the ratios between PC-liposome-treated and LPS treated ones (control) were observed. On the other hand, the i-NOS mRNA band from the extract of macrophages treated with PS-liposomes was faint, and the ratio of i-NOS mRNA to β -actin mRNA showed less than half that of the control (Fig. 4(B)), suggesting that PS-liposomes inhibit NO production at the up-stream of transcription of i-NOS mRNA following stimulation with LPS. These findings correspond well with data of Western blot analysis and of NO production.

Contribution of Scavenger Receptor Negatively charged liposomes composed of anionic phospholipids such as PS are preferentially taken up by phagocytic cells, especially macrophages.²⁷⁻²⁹⁾ Several studies have demonstrated that scavenger receptors (SRs) which bind chemically modified lipoproteins such as oxidized lipoprotein (OxLDL) and acetylated LDL (Acetyl LDL) may in some cases function as anionic phospholipid receptors.^{30,31)} Yang *et al.*,³²⁾ Thai *et al.*³³⁾ and Hamilton *et al.*³⁴⁾ showed the expression of inflam-

matory cytokine mRNAs and release of NO to be inhibited by Ox-LDL in macrophages. These findings suggest that SR is involved in the inhibition of NO production, but the mechanism of this effect remains to be established. Many types of scavenger receptors have been reported, and the relationships between scavenger receptors and ligand binding specificity are shown in Table 2. Thus, we investigated the effects of several ligands for SRs on NO production from macrophages stimulated by LPS to determine the contribution of SR to the regulation of NO production.³⁵⁾

NO production from macrophages treated with Ox-LDL, heparine, and maleylated BSA was inhibited as well as from those treated with PS-liposomes, while no inhibition was observed by native LDL, acetyl-LDL, or dextran sulfate treatment (Fig. 5). Western blot analysis of i-NOS protein revealed that the pretreatment of macrophages with Ox-LDL, heparin, and maleylated BSA also inhibited the induction of i-NOS (Fig. 6). Furthermore, the pretreatment of macrophages with Ox-LDL was required to decrease NO production to control levels as well as PS-liposomes (data not shown).

CD14, a 55 kDa protein on the surface of mononuclear phagocytes, is thought to be a receptor for LPS and is closely related to the signal transduction of LPS for NO production.³⁶⁾ The inhibitory effect of several ligands for SR(s) on NO production induced by LPS would thus appear to be derived from the inhibition of LPS binding to the receptor

Fig. 5. Effects of Several Ligands for SR(s) on NO Production from Macrophages Stimulated with LPS

Macrophages were treated with various ligands at indicated concentrations, and further incubated with LPS (10 μg/ml) for 48 h. The supernatant of macrophage cultures was collected and NO production was evaluated by measuring nitrite. The values are the mean ± S.D. of triplicate cultures from three independent experiments.

Fig. 6. Effects of Several Ligands for SR on i-NOS Expression in Macrophages Stimulated with LPS

Macrophages were treated with the ligands at the concentrations indicated in Fig. 5 for 24 h, and further incubated with LPS (10 μg/ml) for 48 h. Macrophage lysates were subjected to Western blot analysis.

through ligand-LPS complex formation, or ligands may compete for LPS receptor. Pretreatment appears essential to detect the inhibitory activities of Ox-LDL and PS-liposomes on NO production, and no inhibitory effects were observed following concomitant treatment of macrophages with these ligands and LPS. Thus, the inhibition of NO production by several ligands for SR(s) does not appear to be due to the formation of a ligand-LPS complex or PS-liposomes (or Ox-LDL) competing for the LPS receptor; SR(s), especially CD36 and/or macrosialin, are suggested to contribute this inhibition, because of their ligand binding specificity as indicated in Table 2.

Sambrano and Steinberg³⁷⁾ and co-workers³⁸⁾ reported that a plasma membrane protein of 94–97 kDa (macrosialin) was one of the SRs of macrophage for Ox-LDL; PS-liposomes showed strong binding to the protein and this binding clearly inhibited Ox-LDL binding to macrophages. To determine whether macrosialin participates in the inhibitory effects of PS-liposomes on NO production, the effects of anti-macrosialin monoclonal antibody (FA/11, kindly given by Professor S. Gordon, Oxford University, U.K.) on the inhibi-

Fig. 7. Effects of Monoclonal Antibody for Macrosialin (FA/11) on NO Production from Macrophages Stimulated with LPS

Macrophages were incubated for 2 h with FA/11 (100-fold dilution of culture supernatant of hybridoma) and then for another 24 h with PS-liposomes at the indicated concentrations. Macrophages were further incubated for 48 h with or without LPS (10 μg/ml). The supernatants of macrophage cultures were collected and nitrite was measured. The values are the mean ± S.D. ($n=3$).

tion of NO production by PS-liposomes were investigated.³⁹⁾ FA/11 itself showed no effect on NO production induced by LPS. Binding of FA/11 to macrophages was inhibited by the addition of PS-liposomes, indicating that the liposomes have an affinity for macrosialin. However, the inhibitory effects of these ligands on NO production were not reduced even in the presence of FA/11 (Fig. 7). These findings suggest that macrosialin does not contribute to the inhibitory effects of PS-liposomes on NO production from macrophages stimulated with LPS, and the contribution of other type of receptors.

Normally, very little, if any, PS is found on the inner leaflet of the plasma membrane of cells, and the maintenance of this asymmetry is an energy-requiring process attributed to an aminophospholipid translocase located within the plasma membrane.^{40,41)} Early after the initiation of programmed cell death (apoptosis), the loss of phospholipid asymmetry and the exposure of PS on the outer surface of the plasma membrane occur.⁴²⁾ The exposure of PS on the outer surface of apoptotic cells triggers specific recognition and removal by macrophages.^{43,44)} In this recognition, the contributions of different receptors such as the vitronectin receptor,⁴⁵⁾ CD36,⁴⁶⁾ CD14,⁴⁷⁾ and class B scavenger receptor⁴⁸⁾ as well as macrosialin have been reported. Furthermore, Fadok *et al.*⁴⁹⁾ recently cloned a gene that appears to recognize PS on apoptotic cells. Therefore, signal(s) generated following the binding of PS-liposomes to macrophages through these receptors, but not macrosialin, probably interfere with NO production from mouse peritoneal macrophages stimulated with LPS.

Fig. 8. Effects of Liposomes on the Expression of TGF- β mRNA

Macrophages (1×10^6 cells) were treated with PC- or PS-liposomes (500 μ g phospholipid/ml) for indicated times. Total RNAs were extracted and then RT-PCR was performed. Lower panel shows the changes in ratio of TGF- β to β -actin which were evaluated from the band intensity using NIH image.

Inhibitory Mechanism of PS-Liposomes on NO Production

Does TGF- β Act as a Factor That Inhibits the NO Production? Pretreatment of macrophages with PS-liposomes appeared to be required in order to observe the inhibitory activity in NO production induced by LPS as mentioned above; no effect was observed when PS-liposomes were added to macrophage culture at the same time as LPS stimulation. We thus speculated that a factor interfering with NO production could be secreted from macrophages following the PS-liposome treatment. Macrophages were treated with liposomes for 24 h, and culture supernatant was collected by ultracentrifugation. The supernatant was added to the naive macrophages and incubated with LPS for 48 h. NO production induced by LPS was only inhibited by the culture supernatant prepared by incubating the macrophages with PS-liposomes (data not shown). The intensity of inhibition was almost the same as that of the positive control, macrophages that were treated with PS-liposomes directly. These results suggest that macrophages secrete an inhibitory factor and this factor inhibits NO production from macrophages stimulated with LPS.

Apoptotic cells express cell surface changes that allow recognition and removal by macrophages. Removal occurs before lysis, which prevents the release of potentially toxic and immunogenic intracellular contents from the apoptotic cells into the surrounding tissue, and inflammation is avoided. For the removal of apoptotic cells, macrophages recognize PS on these cells and phagocytize them through CD36 as a predominant scavenger receptor.^{50,51} In consequence, macrophages produce transforming growth factor (TGF)- β , prostaglandin E₂ (PGE₂) and platelet-activating factor (PAF), and these products inhibit the production of inflammatory cytokines such as interleukin (IL)-1 β , IL-8, tumor necrosis factor (TNF)- α and granulocyte macrophage-colony stimulating factor (GM-CSF) from macrophages stimulated with LPS. TGF- β is a multifunctional polypeptide growth factor that has been increasingly recognized as an important immunoregulatory molecule, although its reported effects on immunological responses are often contradictory. TGF- β was shown previously to downregulate the production of proinflammatory mediators in macrophages.^{52–54} Therefore, we turned our attention to TGF- β as a factor that would modulate NO production from macrophages following PS-liposome treatment, and its production was evaluated by RT-PCR analysis. Figure 8 shows the expression of TGF- β mRNA following the treatment of macrophages with liposomes.

Fig. 9. Effects of TGF- β on NO Production from LPS-Stimulated Macrophages

Macrophages (1×10^5 cells/ml) were treated with TGF- β at the indicated concentrations for 24 h, and further incubated with LPS (10 μ g/ml) for 48 h. The supernatant of macrophage cultures was collected and NO production was evaluated by measuring nitrite.

some. TGF- β mRNA was constitutively expressed in macrophages, its level began to increase 1 h after the addition of PS-liposomes, and reached maximum at 3 h. No change in TGF- β levels was observed in macrophages treated with PC-liposomes, however, and the same levels as control were shown throughout the experimental period. Furthermore, NO production from macrophages stimulated with LPS was inhibited in a dose dependent manner by the addition of TGF- β (Fig. 9), but this inhibitory effect on NO production was not complete, suggesting the contribution of other factor(s).

There are several reports^{52,53} describing that TGF- β could inhibit NO production from macrophages. Vodovotz *et al.*⁵⁴ also reported the inhibitory activity of TGF- β on NO production from macrophages, and suggested that the potency of TGF- β reflects the suppression of i-NOS expression by three distinct mechanisms, decreased stability and translation of i-NOS mRNA, and increased degradation of i-NOS protein. Our findings obtained in Fig. 4, in contrast, clearly showed that the expression of i-NOS mRNA was inhibited by the treatment of macrophages with PS-liposomes. Studies are now underway to clarify how PS-liposomes affect macrophages to induce TGF- β and how TGF- β inhibits NO production.

Fig. 10. Effects of Liposomes on the Degradation of I- κ B α Induced by LPS Stimulation

Macrophages (1×10^6 cells) were treated with PC-, PS-liposomes (500 μ g phospholipid/ml), or buffer (HBSS: Hanks balanced salt solution) for 24 h, and then stimulated with LPS (10 μ g/ml) for indicated times. Cell lysates were analyzed by Western blot using anti-mouse I- κ B α antibody.

PS-Liposomes Inhibit p38 MAP Kinase, but Not NF- κ B Activation: The details of the signal transduction cascade involved in the induction of i-NOS in response to LPS are an active area of investigation. Although LPS-induced i-NOS induction in macrophages, the molecular mechanism involved in this process is not fully understood.

Nuclear transcriptional factor (NF)- κ B appears to play a primary role in the transcriptional regulation of the i-NOS gene in macrophages.^{55,56} In unstimulated macrophages, NF- κ B is present in the cytoplasm in an inactive form, complexed with NF- κ B inhibitor protein I- κ B. Upon stimulation, I- κ B is phosphorylated on specific serine residues, and degraded in an ubiquitin-dependent process. Then the activated NF- κ B is translocated into the nucleus, binds to the κ B-site on DNA, and induces the expression of i-NOS mRNA. Therefore, we first investigated the effects of PS-liposomes on the degradation of I- κ B α using Western blot analysis. Immediately after the addition of LPS, I- κ B α protein disappeared and then reappeared during incubation (Fig. 10). In the case of macrophages pre-treated with PC- or PS-liposomes, the band intensity of I- κ B exhibits the same profile as that of control pre-treated with buffer solution. These results suggest that PS-liposomes do not interfere with the degradation of I- κ B in macrophages stimulated with LPS.

DNA binding activity of NF- κ B in the nucleus of macrophages was examined by electrophoretic mobility shift assay (EMSA) using 32 P-labeled NF- κ B specific oligonucleotides. PS-liposome treatment did not alter NF- κ B translocation into the nucleus or DNA binding in macrophages stimulated with LPS, and gel shift mobility was the same as that of those treated with LPS (Fig. 11). Consequently, the inhibition of NO production from macrophages treated with PS-liposomes did not result from the inhibition of DNA binding of transcription factor NF- κ B.

In macrophages, LPS stimulates the simultaneous activation of three classes of mitogen activated protein (MAP) kinases: extracellular signal-related kinase (ERK), *c-jun* N-terminal kinase, and p38 with different activation kinetics.⁵⁷ Recently, Carter *et al.*⁵⁸ and others⁵⁹ reported that the p38 MAP kinase regulates NF- κ B-dependent gene transcription by modulating the binding activity of TATA-binding protein (TBP), a basal transcriptional factor, to the TATA box, and the phosphorylation of TBP by the p38 MAP kinase is required for the binding of TBP to the TATA box. Since the p38 MAP kinase activation is regulated by dual phosphorylation on tyrosine and threonine,⁶⁰ the effects of liposomes on the phosphorylation of tyrosine was examined by Western blotting. The phosphorylation of tyrosine induced by LPS stimulation was clearly inhibited by the treatment of macrophages with PS-liposomes, while no inhibition was observed in those which were PC-liposome-treated (Fig. 12).

Fig. 11. Effects of Liposomes and SB203580 on NF- κ B Activation Induced by LPS

Macrophages (1×10^7 cells) were treated with PC- and PS-liposomes (500 μ g phospholipid/ml) for 24 h, and further stimulated with LPS (10 μ g/ml) for 60 min. In the case of SB203580, macrophages were treated for 2 h, then stimulated with LPS. The nuclear proteins were extracted and EMSA analysis of these proteins was conducted using 32 P-labeled oligonucleotide probe containing NF- κ B site.

Fig. 12. Effects of Liposomes on the p38 MAP Kinase Activation Induced by LPS

Macrophages (1×10^6 cells) were treated with liposomes (500 μ g phospholipid/ml) for 24 h, and then stimulated with LPS (10 μ g/ml) for 15 min. Cell lysates were analyzed by Western blot using anti-mouse p38 MAP kinase antibody (lower panel) and anti-phosphotyrosine antibody (upper panel).

To investigate whether p38 MAP kinase is involved in the LPS-stimulated induction of i-NOS in macrophages, we examined the effects of SB203580, a specific inhibitor of this kinase, on NO production from macrophages stimulated with LPS. SB203580 inhibited LPS-stimulated NO induction in a dose dependent manner, and the complete inhibition was observed at 50 μ M (Fig. 13). At the concentrations used, no changes in cell viability were observed (data not shown). Furthermore, the effects of SB203580 on DNA binding of NF- κ B were examined by EMSA, and was found no to alter

Fig. 13. Effects of SB203580 on NO Production from Macrophages Stimulated with LPS

Macrophages (1×10^5 cells/ml) were treated with SB203580 at indicated concentrations for 2 h, then further incubated with LPS ($10 \mu\text{g/ml}$) for 48 h. The supernatant of macrophage cultures were collected and NO production was evaluated by the measurement of nitrite.

DNA binding of NF- κ B by the evaluating gel shift mobility (Fig. 11), indicating that the p38 MAP kinase may not be involved in the process of NF- κ B activation or DNA binding of NF- κ B. From these results, one possible mechanism for the inhibitory effects of PS-liposomes on NO production from LPS-stimulated macrophages might be the inhibitory effects of the liposomes on p38 MAP kinase activation. Studies are currently underway to learn how PS-liposome treatment interferes with the p38 MAP kinase activation in macrophages stimulated with LPS.

CONCLUSION

The effects of liposomes on NO production from LPS-stimulated macrophages were examined, and negatively charged liposomes composed of phosphatidylserine were found to inhibit the production. The proposed inhibitory mechanism of PS-liposomes on NO production is illustrated in Fig. 14: following treatment with these liposomes, macrophages secrete a factor which interferes with i-NOS expression induced by LPS, and TGF- β appears to be a candidate for this factor. PS-liposome treatment did not inhibit transcriptional factor NF- κ B activation which appears to play a primary role in the transcriptional regulation of the i-NOS gene in macrophages. The p38 MAP kinase is essential to the LPS-induced i-NOS gene expression, and understood regulates NF- κ B-dependent gene transcription by modulating activation of the TATA box binding protein.⁵⁸⁾ Thus, a factor secreted from macrophages by treatment with PS-liposomes might inhibit the p38 MAP kinase activation induced by LPS treatment.

The exposure of PS on the outer surface of apoptotic cells triggers the removal by macrophages. Removal occurs before lysis, and inflammation is avoided. The mechanism is not yet fully understood, but secretions of TGF- β , PGE₂ and PAF which inhibit the production of inflammatory cytokines from macrophages are indicated.^{50,51)} Consequently, PS-liposomes might be a suitable model for understanding the changes in

Fig. 14. Schematic Representation of Inhibitory Mechanism of PS-Liposomes on NO Production from Macrophages Stimulated with LPS

macrophage functions induced by the phagocytosis of apoptotic cells. Furthermore, since macrophages preferentially take up liposomes and macrophages migrate where inflammation occurs, PS-liposomes could be a candidate as DDS carrier for anti-inflammatory drugs, expecting the anti-inflammatory effects of PS-liposomes themselves and the encapsulating drugs.

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