Regulation of the Surface Expression of the Platelet-activating Factor Receptor in IC-21 Peritoneal Macrophages

EFFECTS OF LIPOPOLYSACCHARIDE*

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The effect of bacterial lipopolysaccharide (LPS) on the expression of the receptor for platelet-activating factor (1-O-alkyl-2-acetyl-sn-glycero-3-phosphocholine; AGEPC) was examined in cultured IC-21 peritoneal macrophages. AGEPC binding to its receptors reached saturation within 20 min at 25 °C and was reversible. Scatchard analysis revealed a single class of AGEPC receptors with a Bmax of approximately 170 fmol/mg cellular protein and a Kd of 0.25 nM. Preincubation of IC-21 cells with LPS (0.01–1,000 ng/ml) induced an increase in the surface expression of AGEPC receptors in a time- and concentration-dependent fashion. The maximal effect of LPS on the AGEPC receptor was observed between 5 and 8 h, with a typical increase between 150 and 200%. Scatchard analysis indicated that LPS treatment of IC-21 cells increased the number of AGEPC receptors on the cell surface without any apparent change in the affinity of the receptor for the ligand. The effect of LPS on the surface expression of the AGEPC receptor was nearly abolished by cycloheximide (0.1 mM) and by actinomycin D (3 μM), suggesting the involvement of enhanced receptor protein synthesis and mRNA production in this event. Moreover, LPS treatment increased the capability of the IC-21 cell to respond to AGEPC addition by elevating intracellular free Ca2+ without causing an increase in the basal level of intracellular Ca2+. The present study demonstrates that IC-21 peritoneal macrophages possess high affinity AGEPC receptors and provides the evidence that the number of functional AGEPC receptors on a cell can be increased significantly upon exposure to LPS.

Platelet-activating factor or 1-O-alkyl-2-acetyl-sn-glycero-3-phosphocholine (AGEPC)1 is a biologically active phospholipid (1–3) which elicits most of its biological effects via specific receptors which have been identified in various tissues and cells, including platelets, neutrophils, monocytes, and macrophages (4–10). These cells respond to stimulation by AGEPC to exhibit various physiological and pathophysiological effects including induction of platelet aggregation and secretion (1, 11), chemotaxis and degranulation of polymorphonuclear leukocytes (12), intracellular calcium mobilization in macrophages (13, 14), and arachidonic acid release and cyclooxygenase-derived eicosanoid production in rat Kupffer cells (10).

Bacterial lipopolysaccharide (LPS), a major surface component of the cell wall of Gram-negative bacteria, is responsible for various pathophysiological and biochemical events in intact animal systems, including the induction of the endotoxin shock syndrome and activation of the immune system and the complement cascade (15). Under certain pathophysiological conditions, LPS is absorbed from the gastrointestinal tract in significant amounts because of altered permeability of the intestinal mucosa. Alternatively, LPS may be derived directly from Gram-negative microorganisms during episodes of bacterial sepsis. The primary physiological responses of a host animal to endotoxin are believed to take place in reticuloendothelial cells, particularly macrophages. The interaction of endotoxin with macrophages is likely one of the most important features of inflammation or the shock response following bacterial invasion. Macrophages play an important role in inflammation and host defense against bacterial infection (16, 17). In fact, it has been shown that many of the actions of LPS are mediated by macrophages which may produce important lipid mediators including prostaglandins, leukotrienes, and platelet-activating factor (18–20). These observations suggest a possible functional interaction between LPS and various mediators such as platelet-activating factor and require exploration of the molecular mechanisms involved in such interactions. A recent study has demonstrated that LPS is able to prime macrophages for enhanced arachidonic acid metabolism increasing AGEPC-stimulated prostaglandin E2 production (21). The mechanism for the LPS priming of the AGEPC-stimulated response was not definitively characterized. LPS priming may occur through several mechanisms: (a) an increase in the ligand affinity and/or the number of AGEPC receptors or the rearrangement of AGEPC receptors on the cell surface; (b) modification of postreceptor signaling events; (c) modification of the effector systems such as the enzymatic activity responsible for arachidonic acid metabolism. Hence, it seemed important to investigate whether LPS exerts regulatory effects on AGEPC receptors in macrophages.

In the present investigation, we have demonstrated (a) that IC-21 peritoneal macrophages possess receptors with a high affinity for AGEPC and (b) that LPS challenge increases the surface expression of AGEPC receptors and consequently...

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1 The abbreviations used are: AGEPC, 1-O-alkyl-2-acetyl-sn-glycero-3-phosphocholine; LPS, lipopolysaccharide; lypoAGEPC, 1-O-alkyl-2-[3-O-sulfate]-sn-glycero-3-phosphocholine; BSA, bovine serum albumin; WEB2170, [8(R,S)-6-(2-chlorophenyl)-8,9-dihydro-1-methyl-8-(4-morpholinylcarbonyl)-4H,7H-cyclopenta(4,5)thieno[3,2-f][1,2,4]triazolo[4,3-e][1,4]diazepine; [Ca2+]i, concentration of intracellular free calcium; LBP, lipopolysaccharide-binding protein.

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enhances AGEPC receptor-mediated transmembrane signaling, e.g. an increase in cytosolic free calcium.

**EXPERIMENTAL PROCEDURES**

**Materials**—[**H**-hexadecyl]AGEPC, 40.5-59.5 Ci/mmol and [ace
ty]l-[**H**]AGEPC, 10.0 Ci/mmol) were purchased from Du Pont New England Nuclear. Unlabeled AGEPC(C$_{n}$so) was purchased from BACHEM BioScience, Inc. (Torrance, CA). Lipopolysaccharide (Escherichia coli 055:B5), forskolin, dibutyryl cAMP, digitonin, and EGTA were purchased from Sigma. Prostaglandin D$_{2}$ was obtained from Advanced Magnetics Inc. (Cambridge, MA). Cycloheximide and actinomycin D were purchased from Calbiochem. Fura-2/AM and pluronic acid were purchased from Molecular Probes Inc. (Eugene, OR). WEB2170 was a gift from Boehringer Ingelheim (Ridgefield, CT).

**Cell Culture**—IC-21 peritoneal macrophages were obtained from the American Type Culture Collection (Bethesda, MD) and maintained in RPMI 1640 culture medium ( Gibco) supplemented with 10% heat-inactivated (56 °C, 30 min) fetal bovine serum (HyClone Laboratories, Logan, UT), 112 units/ml penicillin, and 112 units/ml streptomycin at 37 °C in a humidified atmosphere of 90% air, 10% CO$_{2}$. Adherent cells were passaged every 5-6 days into 35-mm Petri dishes at a density of 1 x 10$^{6}$ cells/plate. The subcultured cells were used 2 days following passage.

[**H**]AGEPC Binding to IC-21 Peritoneal Macrophages—Adherent cells in 35-mm Petri dishes were rinsed three times with Hanks' buffer containing 0.1% BSA, 0.5 mM MgCl$_{2}$, and 5 mM CaCl$_{2}$, and incubated with [**H**]AGEPC at 25 °C in the absence or presence of a 1,000-fold excess of unlabeled AGEPC. After the time periods indicated, the binding reaction was terminated by removing Hanks' buffer and rinsing the cells with ice-cold phosphate-buffered saline containing 0.1% BSA. Cells were lysed in 0.5 ml of 1 M NaOH, and radioactivity was determined in a scintillation counter. Specific [**H**]AGEPC binding is defined as the difference between total binding (measured in the absence of 1.0 μM unlabeled AGEPC) and nonspecific binding (measured in the presence of 1.0 μM unlabeled AGEPC).

In dissociation experiments, cells were incubated with [**H**]AGEPC in the presence of labeled ligand for 30 min followed by the addition of 4 μM unlabeled AGEPC or unlabeled WEB2170. At each time point, [**H**]AGEPC binding was terminated as indicated above.

**Quantitation of Protein**—Protein was quantitated using the bicinchoninic acid protein assay essentially as described by Smith et al. (25). The reagent kits employed in this assay were provided by Pierce Chemical Co. BSA was used in the construction of a standard curve. Protein was quantitated by measuring the absorbance of the sample at 562 nm.

**Quantitation of Intracellular Calcium Concentrations**—Measurements of intracellular calcium were performed using the fluorescent indicator Fura-2/AM (0.1% BSA, 0.5 mM MgCl$_{2}$, and 5 mM CaCl$_{2}$), and incubated with [**H**]AGEPC at 37 °C in the presence of 0.25% pluronic acid in serum-free RPMI 1640 medium, pH 7.4. After 60 min the coverslips were washed with calcium-free Hanks' buffer and incubated for 20 min at 37 °C with 0.1% BSA. Cells were lysed in 0.5 ml of 1 M NaOH, and radioactivity was determined in a scintillation counter. The fluorescence ratio of 340/380 nm at zero and saturating Ca$^{2+}$ concentration; $V$ is the ratio of the fluorescence at 380 nm at zero and saturating Ca$^{2+}$ concentration; $K_{d}$ is the dissociation constant of Fura-2/AM-Ca$^{2+}$ complex, i.e. 224 nM.

**RESULTS**

**Characterization of Specific [**H**]AGEPC Binding in IC-21 Peritoneal Macrophages**

**Metabolism of AGEPC in IC-21 Cells**—To interpret AGEPC receptor binding data appropriately, it was important to ascertain whether the AGEPC molecule remained intact under the incubation conditions of the binding studies. [**H**-hexadecyl]AGEPC was added to cultured IC-21 cells in Hanks' buffer containing 0.1% BSA and incubated at 25 °C for various time periods. As shown in Table I, the metabolism of AGEPC was negligible under the binding conditions; more than 96% of the added [**H**-hexadecyl]AGEPC remained intact after 60 min of incubation. Similarly, there was minimal AGEPC metabolism in LPS-treated cells (Table I). To examine the possible activity of acetylhydrolase, the production of free [**H**]acetate from [**H**-acetyl]AGEPC was measured in cultured IC-21 cells. The results shown in Table II indicate that 11.5 fmol (1.1% of the total added) of [**H**]acetate was released from cells within 30 min and 20.5 fmol (1.9% of the total added) within 60 min. These results suggest that the metabolism of AGEPC in IC-21 cells was minimal in the binding experiments.

**Time Course of [**H**]AGEPC Binding**—IC-21 murine peritoneal macrophages (1 x 10$^{6}$ cells/plate) were incubated with 1.0 nM [**H**]AGEPC at 25 °C for time periods from 1 to 60 min followed by specific [**H**]AGEPC binding assessment. As shown in Fig. 1, specific [**H**]AGEPC binding to IC-21 cells was rapid during the initial 10 min and reached a steady-state level of binding within 20 min. The maximal amount of [**H**]AGEPC specifically bound to the IC-21 peritoneal mac-

| Time (min) | [**H**]Lysate | [**H**]AGEPC | [**H**]AlkylacylGPC* |
|-----------|--------------|--------------|---------------------|
| min       | radioactivity recovered | % of total recovered | % of total recovered |
| Control cells | 0 | 1.4 | 98.4 | 0.3 |
| 30 | 1.86 ± 0.88 | 97.0 ± 0.69 | 1.06 ± 0.6 |
| 60 | 2.5 ± 0.4 | 95.2 ± 1.1 | 2.35 ± 1.5 |
| LPS-pretreated cells | 30 | 1.25 | 97.6 | 1.2 |

* AlkylacylGPC, 1-O-alkyl-2-acetyl-sn-glycero-3-phosphocholine.
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**TABLE II**

Metabolism of [3H-acetyl]AGEPC by IC-21 peritoneal macrophages

| Time (min) | [3H]Acetate released (fmol) | % of radioactivity added |
|-----------|-----------------------------|-------------------------|
| 0         | 64.7 ± 1.9                  | 6.2 ± 0.1               |
| 30        | 76.2 ± 7.4                  | 7.3 ± 0.7               |
| 60        | 85.2 ± 4.2                  | 8.1 ± 0.4               |

Time course of [3H]AGEPC binding to IC-21 peritoneal macrophages. 1.0 nM [3H]AGEPC was incubated with 1 × 10^6 cells at 25 °C for the indicated time periods followed by specific [3H]AGEPC binding assessment. The specific AGEPC binding (○) was determined as described under "Experimental Procedures." O, nonspecific binding; A, total binding.

Equilibrium analysis of [3H]AGEPC binding—[3H]AGEPC at various concentrations (from 0.1 to 2.25 nM) was incubated with IC-21 cells at 25 °C for 30 min to attain an equilibrium state in the presence or absence of excess unlabeled AGEPC followed by specific AGEPC binding assessment. The results in Fig. 2 indicate that specific [3H]AGEPC binding was concentration-dependent and saturable. In contrast, the nonspecific binding of [3H]AGEPC was nonsaturable. Scatchard analysis suggested that the binding data were best fitted to a model of one binding class (Fig. 2, inset). The equilibrium dissociation constant, K_d, for the interaction of [3H]AGEPC with the specific binding sites was 0.25 nM, and the B_max was approximately 170 fmol/mg protein (Fig. 2, inset).

LPS Regulation of the AGEPC Receptor in IC-21 Peritoneal Macrophages

Effect of Various Compounds on AGEPC Receptor Expression—To investigate the receptor regulatory effect of LPS, we examined initially the effect of several compounds including dibutyryl-cAMP, forskolin, prostaglandin D_2, and LPS on specific [3H]AGEPC binding. Cells were pretreated with LPS (100 ng/ml), dibutyryl-cAMP (1.0 mM), forskolin (0.1 mM), and prostaglandin D_2 (1 μM) at 37 °C for 20 h followed by an assessment of [3H]AGEPC binding. As shown in Fig. 3, prostaglandin D_2 had no effect on specific [3H]AGEPC binding. Long term incubation of IC-21 cells with dibutyryl-cAMP or forskolin, on the other hand, down-regulated specific AGEPC binding by 50%. LPS treatment of IC-21 cells, in contrast, led to nearly a 150% increase in specific AGEPC binding (Fig. 3).

Effect of LPS on Surface Expression of the AGEPC Receptor: Concentration and Time Dependence—Cells were treated with various concentrations of LPS for 5 h at 37 °C followed by a washing procedure and measurement of specific [3H]AGEPC binding. As shown in Fig. 4A, LPS treatment increased specific [3H]AGEPC binding in a concentration-dependent fashion. The "up-regulatory" effect of LPS was observed at con-
The effect of LPS was time-dependent and somewhat transient. As shown in Fig. 4B, up-regulation of AGEPC binding occurred rapidly during the initial periods between 1 and 5 h after the addition of LPS (100 ng/ml) and reached a peak level within 5 h. The up-regulated \([{}^{3}H\)]AGEPC binding decreased gradually after 8 h and was maintained at approximately 140% of control between 20 and 24 h.

**Equilibrium Analysis of \([{}^{3}H\)]AGEPC Binding in LPS-treated IC-21 Cells**—To determine whether the LPS-induced increase in specific \([{}^{3}H\)]AGEPC binding was caused by an increase in AGEPC binding affinity or an increase in the number of specific AGEPC binding sites, the equilibrium of specific AGEPC binding was analyzed in control and LPS-treated IC-21 cells. As shown in Fig. 5A, \([{}^{3}H\)]AGEPC binding increased by nearly 1-fold at almost all concentrations of \([{}^{3}H\)]AGEPC in LPS-treated cells as compared with untreated cells. Scatchard analysis indicated that the \(K_d\) value (0.25 nM) did not change (Fig. 5B). These results suggested that the AGEPC receptor activity in IC-21 peritoneal macrophages was modulated by a change in receptor number on the surface of the macrophage, without any apparent change in the affinity of the receptor for the ligand.

**Reversibility of \([{}^{3}H\)]AGEPC Binding in LPS-treated Cells**—To ascertain that the LPS-induced increase in \([{}^{3}H\)]AGEPC binding was caused by an increase in surface \([{}^{3}H\)]AGEPC binding rather than an enhanced ligand internalization or

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**Fig. 3. Effect of various compounds on specific \([{}^{3}H\)]AGEPC binding.** Cells were either left untreated or treated with LPS (100 ng/ml), dibutyryl (DB) cAMP (1.0 mM), forskolin (0.1 mM), or prostaglandin \((PG)\) D\(_2\) (1 \(\mu\)M) at 37 °C for 20 h followed by a washing procedure and \([{}^{3}H\)]AGEPC binding assay as described under "Experimental Procedures." Results are expressed as the percent of specific \([{}^{3}H\)]AGEPC binding in control cells which received no pretreatment. *\(p < 0.05\) versus control. Each data point represents the mean value ± S.D. of three separate experiments, each performed in duplicate.

**Fig. 4. Concentration and time dependence of LPS-induced increase in specific \([{}^{3}H\)]AGEPC binding.** Cells were either incubated with various concentrations of LPS for 5 h (A) or with 100 ng/ml LPS for various time periods (B). The cells were washed three times with Hanks' buffer, and the \([{}^{3}H\)]AGEPC binding experiments were performed. Results are expressed as the percent increase of specific \([{}^{3}H\)]AGEPC binding in the LPS-treated cells compared with untreated cells. Each data point represents the mean ± S.D. of four separate experiments, each performed in duplicate.

**Fig. 5. Equilibrium analysis of specific \([{}^{3}H\)]AGEPC binding in LPS-treated cells as compared with control.** Cells were preincubated in the presence (●) or absence (○) of LPS (100 ng/ml) in culture medium at 37 °C for 5 h followed by a washing procedure. The binding assay was conducted by incubating the cells with various concentrations of \([{}^{3}H\)]AGEPC at 25 °C for 30 min. Each data point is the mean of duplicate determinations. A, saturation curve; B, Scatchard plot.
metabolism, the reversibility of the bound [3H]AGEPC was examined in LPS-treated cells. The rationale for this type of experiment was that intact surface-bound AGEPC but not AGEPC metabolites or internalized AGEPC would be displaced by an excess unlabeled AGEPC or receptor antagonist. As shown in Fig. 6, 5-h treatment of cells with LPS led to an increase in [3H]AGEPC binding. The specifically bound [3H]AGEPC in control cells and in LPS-treated cells was dissociated rapidly within 30 min following the addition of excess unlabeled WEB2170 (Fig. 6A) or AGEPC (Fig. 6B). The specifically bound ligand was dissociated completely within 60 min after the addition of unlabeled WEB2170. These data suggest that the bound [3H]AGEPC remained intact and was not internalized either in the control cells or in LPS-treated cells under the incubation conditions of the binding experiment.

Serum Dependence of the Receptor Regulatory Effect of LPS—When IC-21 cells were treated with LPS for 5 h in the presence of fetal bovine serum, the specific [3H]AGEPC binding increased by more than 200% of control. This stimulatory effect of LPS was attenuated significantly when cells were treated with LPS in the absence of fetal bovine serum. The absence of serum during treatment also decreased the basal level of specific [3H]AGEPC binding (data not shown). The receptor regulatory effect of LPS was not observed at 24 h when cells were treated with serum-free culture medium (data not shown).

Effect of Actinomycin D and Cycloheximide on the LPS-induced Increase in Surface Expression of the AGEPC Receptor—To investigate the molecular basis for the LPS-induced increase in the surface expression of AGEPC receptors, the effects of actinomycin D, an inhibitor of DNA transcription, and cycloheximide, an inhibitor of translation, on the LPS-induced increase in AGEPC receptors were examined. Cells were incubated with LPS for 2 h at 37 °C followed by the addition of actinomycin D or cycloheximide to the medium, and the incubation was continued for another 4 h. As shown in Fig. 7, LPS treatment caused a time-dependent increase in specific [3H]AGEPC binding in IC-21 cells. The addition of actinomycin D (Fig. 7A) or cycloheximide (Fig. 7B) at an early point in the experiment prevented the occurrence of the LPS-induced increase in specific [3H]AGEPC binding. Both compounds had no effect on the basal [3H]AGEPC binding in control cells under these experimental conditions (data not shown).

AGEPC Receptor-mediated Cytosolic Calcium Responses in IC-21 Peritoneal Macrophages

Effect of AGEPC on Intracellular Calcium Levels—To measure [Ca²⁺], levels, peritoneal macrophages adhering to glass coverslips were incubated with Fura 2/AM at 37 °C for 1 h followed by fluorometric measurement of intracellular free Ca²⁺ as described under “Experimental Procedures.” As shown in Fig. 8A, in the presence of 2 mM extracellular Ca²⁺, AGEPC at a concentration of 10⁻⁶ M induced a rapid and
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Fig. 8. AGEPC-induced increase in intracellular calcium. A. $[Ca^{2+}]_i$, response to $10^{-4}$ M AGEPC in the presence of extracellular $Ca^{2+}$ (2 mM). B. $[Ca^{2+}]_i$, response to $10^{-4}$ M AGEPC in Ca$^{2+}$-free buffer containing 2 mM EGTA. C. effect of LPS (100 ng/ml), lysoGEPC (2 x $10^{-7}$ M), and AGEPC (10$^{-7}$ M) on $[Ca^{2+}]_i$. D. effect of WEB2170 on the AGEPC-induced increase in $[Ca^{2+}]_i$. 2 x $10^{-6}$ M WEB2170 was added to buffer containing 2 mM Ca$^{2+}$ prior to AGEPC (10$^{-9}$ M) stimulation.

transient increase in $[Ca^{2+}]_i$. $[Ca^{2+}]_i$ increased from a basal level of 20 to 150 nM within 30 s and decreased gradually thereafter. To examine the role of extracellular calcium in the AGEPC-induced increase in $[Ca^{2+}]_i$, EGTA was added to "calcium-free" Hanks' buffer prior to AGEPC stimulation. As shown in Fig. 8B, the ability of AGEPC to increase $[Ca^{2+}]_i$ was nearly, if not completely, attenuated in the absence of extracellular free Ca$^{2+}$. An increase in $[Ca^{2+}]_i$ was not observed in cells to which either lysoGEPC or LPS was added (Fig. 8C). WEB2170, an AGEPC receptor antagonist, abolished the AGEPC-induced increase in $[Ca^{2+}]_i$. (Fig. 8D).

Effect of LPS on the AGEPC-induced Increase in Intracellular Level of Calcium—Since incubation of IC-21 cells with LPS led to up-regulation of surface expression of functionally active AGEPC receptors, it was important to determine whether an AGEPC receptor-mediated biological response was enhanced. Fig. 9 shows the effect of LPS treatment on the concentration dependence of the AGEPC-induced net increase in $[Ca^{2+}]_i$. The calcium response to AGEPC in both control cells and LPS-treated cells was concentration-dependent (Fig. 9). The half-maximum response ($EC_{50}$) in control cells was observed at approximately 5 x $10^{-11}$ M AGEPC, which was very close to the $K_d$ value of 0.25 nM in the ligand binding assay. As compared with control cells, the intracellular calcium levels in LPS-treated cells in response to AGEPC increased rapidly to a significantly higher level. The $EC_{50}$ was observed at 2 x $10^{-11}$ M AGEPC. The net increase in $[Ca^{2+}]_i$, in the LPS-treated cells in response to all concentrations of AGEPC tested was 150–200% of that in the control cells. The basal level of intracellular calcium in LPS-treated cells was not changed (16.3 ± 8.4 nM, n = 12 versus 16.4 ± 7.1 nM, n = 14, in control cells).

DISCUSSION

The present investigation demonstrates that the murine IC-21 peritoneal macrophage cell line possesses high affinity, functional AGEPC receptors. The biological activity of these AGEPC receptors was demonstrated by examining the ability of stimulated receptors to elevate intracellular calcium fluxes. $[^3H]$AGEPC binding to its receptor was rapid, saturable (within 20 min at room temperature), and reversible. Scatchard analysis revealed a single class of AGEPC receptors with high affinity for AGEPC. The dissociation constant, $K_d$, derived from equilibrium analysis, was determined to be 0.25 nM, and the calculated density of the binding sites was 170 fmol/mg of cellular protein. The binding affinity of the AGEPC receptor in the murine IC-21 peritoneal macrophage is comparable to that observed in other cell types including rat Kupffer cells (10), P388D1 macrophages (6), neutrophils (9), and platelets (5, 8). To examine whether or not the lipophilic ligand AGEPC was translocated and/or metabolized under the conditions of the ligand binding assay, $[^3H]$AGEPC metabolism at room temperature (25 °C) and dissociation of the bound $[^3H]$AGEPC were examined. The results from both types of experiments indicated that metabolism of $[^3H]$AGEPC was minimal as $[^3H]$AGEPC remained intact and
or agonists. Whereas LPS up-regulated the surface expression of AGEPC receptors, dibutyryl-CAMP and forskolin down-regulated the AGEPC receptor under the same experimental conditions. The regulatory responses to different compounds other than LPS were defined in the present study. The LPS up-regulation of AGEPC receptors was specific for LPS as zymosan (100 ng/ml), an alloendothelial cells, failed to exhibit a modulatory effect on LPS-treated cells indicated that LPS modulated AGEPC receptor activity primarily by increasing the number of expressed receptors without an apparent change in the ligand binding affinity.

Several characteristics of the regulation of AGEPC receptors were defined in the present study. The LPS up-regulation response was specific for LPS as zymosan (100 ng/ml), another particulate which, like LPS, is endocytosed by reticuloendothelial cells, failed to exhibit a modulatory effect on the AGEPC receptor under the same experimental conditions (data not shown). The AGEPC receptor in IC-21 macrophages showed specific regulatory responses to different compounds or agonists. Whereas LPS up-regulated the surface expression of AGEPC receptors, dibutyryl-CAMP and forskolin down-regulated the expression of AGEPC receptors, and prostaglandin D₂ had no effect. The LPS-induced increase in AGEPC receptors appeared to be specific for the AGEPC receptor as LPS did not modulate the surface expression of receptors for radiolabeled phorbol ester (data not shown). In fact, in other studies LPS has been shown to up-regulate interleukin-2 receptors in human monocytes (31, 32) and down-regulate receptors for macrophage/colony-stimulating factor, insulin, and tumor necrosis factor in mouse macrophages (33–35).

The receptor regulatory effect of LPS observed in the present study is potentially important. In a previous study it was demonstrated that LPS is relatively ineffective in stimulating the release and metabolism of arachidonic acid in the macrophage. However, LPS was able to prime these cells for enhanced arachidonic acid release in response to AGEPC (21) and various other stimuli such as zymosan, phorbol 12-myristate 13-acetate immune complexes, calcium ionophore A23187, and latex beads (27). The molecular mechanisms involved in LPS priming of macrophages to release arachidonic acid or to stimulate other cellular responses such as superoxide anion release are not fully understood. Modulation of cellular responses by LPS priming could alter any one of three elements involved in receptor-mediated signal transduction, i.e., the receptor-ligand interaction, the subsequent signal transduction system (e.g., phospholipases, Ca²⁺, cAMP, or tyrosine kinase), or several effector systems (e.g., various proteins and other affected enzyme systems). It was proposed in an earlier study that LPS priming involves alterations in the signal transduction pathway distal to receptor-ligand binding since responses to the binding of zymosan, IgG-opsonized erythrocytes, and latex microspheres remain unchanged in LPS-treated cells (27). In fact, elevated resting intracellular free Ca²⁺ (41) and myristoylation of a protein kinase C substrate (42) in response to LPS priming have been proposed to be involved in the mechanisms for enhanced superoxide anion production and arachidonic acid release, respectively. The finding that LPS priming has no effect on the activity of the NADPH oxidase responsible for production of superoxide anion (41) and phospholipase A₂ leading to release of arachidonic acid (21) suggests that these effector systems may not be targets of LPS priming. In the case of the AGEPC receptor system, LPS priming which enhances arachidonic acid metabolism is believed to be associated with the events of DNA transcription leading to production of effector enzymes involved in the enhanced arachidonic acid metabolism (21). The present results provide a clear evidence that LPS priming of AGEPC-induced biological responses involves modulation of surface AGEPC receptor expression. To ascertain whether LPS treatment had a functional priming effect, AGEPC-induced intracellular calcium fluxes were examined in LPS-treated cells compared with untreated cells. In untreated cells AGEPC induced a dose-dependent increase in intracellular calcium. LPS-treated cells responded to AGEPC with a similar sensitivity but to a greater extent, as evidenced by a similar EC₅₀ value and a greater maximal effect of the AGEPC-induced elevation of the intracellular calcium level. It is likely that LPS primes the AGEPC-induced increase in intracellular calcium fluxes and possibly several other cellular responses subsequent to the increase in [Ca²⁺], (e.g., arachidonic acid release and eicosanoid production and superoxide anion release) through a mechanism involving both an increase in the expression of AGEPC receptors and the sensitizing of some aspect of postreceptor signal transduction.

The molecular details of the AGEPC receptor regulation and functional priming effect of LPS are not fully understood. It has been proposed that LPS acts through its specific...
binding protein on the cell surface to trigger the initial responses of the macrophage. A specific LPS-binding protein has been described in monocytes and in cultured macrophage-like cells (36, 37). Results from the present experiments employing cycloheximide and actinomycin D indicate that LPS increases AGEPC receptor expression through a mechanism involving DNA transcription and receptor protein synthesis. It is likely that LPS, through its specific receptor and a subsequent signal transduction mechanism yet to be defined, modulates biosynthesis of AGEPC receptor protein either at the level of transcription or translation leading to an increase in the surface expression of the receptor. Supporting this proposal is the observation that in rat Kupffer cells, the resident macrophage in the liver, the recovery of down-regulated AGEPC receptors in AGEPC-pretreated cells is dependent upon new protein synthesis (29) and is sensitive to actinomycin D.2 The effect of LPS on the regulation of gene expression has been reported previously; LPS treatment leads to an elevation of mRNA level for proto-oncogenes, c-fos and c-myc, and competence genes JE and KC in peritoneal macrophages (40). LPS priming in macrophage P388D1 cells enhances arachidonic acid metabolism in response to AGEPC, and this effect may involve an alteration of DNA transcription (21). It is noteworthy that the receptor regulatory effect of LPS requires the presence of serum during the priming period. The mechanism of this effect of serum is presently unclear. There are two plausible explanations for the serum dependence of the LPS effect. (a) In normal serum there exists a small amount of lipopolysaccharide-binding protein (LBP) (43) which has been found to bind to the lipid A moiety of LPS and to enhance the effect of LPS greatly (44, 45). LBP is believed to bridge LPS particles to macrophages by binding directly to the LPS and then to the macrophage. Therefore, the presence of fetal bovine serum containing LBP could facilitate the binding LPS to cultured IC-21 macrophages and subsequently enhance the priming effect of LPS. In fact, a recent study has shown that LBP enhances LPS priming of neutrophils for formyl peptide-induced superoxide-anion production (45) and that LPS-LBP complexes are as much as 1,000-fold more active than LPS alone in the induction of the synthesis of monokines such as tumor necrosis factor or interleukin-1β (22). (b) Serum contains various growth factors and co-factors which are necessary for protein synthesis as well as cell growth. Lack of such elements in the medium may affect the mechanisms by which AGEPC induces homologous desensitization of AGEPC receptors. The present finding that LPS up-regulates AGEPC receptors is novel and adds an important detail to the mechanism(s) by which LPS-autocoid (e.g. AGEPC derived from macrophages or other host inflammatory cells) interactions occur in several pathophysiological situations such as bacterial sepsis and endotoxin-induced shock. This LPS-autocoid receptor regulatory mechanism likely increases the effectiveness of macrophages in host defense and could, in fact, potentiate tissue injury initiated by bacterial endotoxin.

In summary, the present study demonstrates that the IC-21 peritoneal macrophage possesses a large number of AGEPC receptors. Exposure of these cells to LPS leads to an increased expression of the AGEPC receptors. The receptor regulatory effect of LPS is time- and concentration-dependent, ligand-specific, and serum-dependent. The mechanism by which LPS induces an increase in surface expression of AGEPC receptors involves enhanced receptor mRNA production and protein synthesis. Additionally, LPS priming induces an enhanced intracellular free Ca++ response to AGEPC.

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