Vol. 165, No. 1, 1989 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
November 30, 1989 Pages 526-532

SENSITIZATION OF ALVEOLAR MACROPHAGES TO LIPOPOLYSACCHARIDE-INDUCED PROSTAGLANDIN SYNTHESIS BY EXOGENOUS PROSTAGLANDINS

Ken-ichi Tanamoto*, Ulrich Schade and Ernst Th. Rietschel

Forschungsinstitut Borstel,
D-2061 Borstel, FRG

Received October 23, 1989

Rabbit alveolar macrophages were found to produce extraordinary amounts of prostaglandin E_2 and $F_2\alpha$ with the stimulation of lipopolysaccharide or lipid A. Exogenous prostaglandin E_2 greatly enhanced the lipopolysaccharide action on rabbit alveolar macrophages for the induction of prostaglandin $F_2\alpha$ release (3-5 fold), while prostaglandin E_2 alone did not cause any effect. The enhancement expressed was especially strong when prostaglandin E_2 was administered to the cells simultaneously with lipopolysaccharide. The effect of prostaglandin E_2 was observed neither with a nonstimulating dose of lipopolysaccharide nor with a stimulating dose of zymosan. This phenomenon was even more pronounced when prostaglandin I_2 was used instead of prostaglandin E_2, while no sensitization was demonstrated by prostaglandin $F_2\alpha$. These observations suggest that prostaglandins can modulate the activation of the cyclooxygenase pathway of arachidonate metabolism in the activated macrophages by lipopolysaccharide.

Prostaglandin E group (E_1 and E_2) has been found to inhibit various macrophage functions such as adhesion, migration and phagocytosis of mouse peritoneal macrophages (1,2) and production of plasminogen activator (3). In contrast prostaglandin E_2 (PGE_2) enhances collagenase production in guinea pig macrophages stimulated by LPS (4). There are some suggestions of evidence for prostaglandin mediation of some of the biological activities of endotoxin. Thus, an inhibitor of prostaglandin synthesis such as indomethacin or aspirin protects from some of the endotoxic effects (5,6). It is known that lipopolysaccharide (LPS) can stimulate mononuclear phagocytes inducing a high enough level of prostaglandin production for the manifestation of LPS effects (6-8). These facts and others lead us to question whether prostaglandins have a regulatory effect on the activity of LPS. Here we report that the elevated levels of PGE_2 produced by endotoxin-stimulated rabbit

* To whom correspondence should be addressed. Present address: Department of Microbiology, National Institute of Hygienic Sciences, Kamiyoga 1-18-1, Setagaya-ku, Tokyo 158, Japan.

Abbreviations: PGE_2 and $F_2\alpha$, prostaglandin E_2 and $F_2\alpha$; LPS, lipopolysaccharide.

0006-291X/89 $1.50

alveolar macrophages have a regulatory role in the induction of cyclooxygenase products by these cells.

MATERIALS AND METHODS

Materials: Lipopolysaccharide was obtained from Citrobactor and lipid A from S.minnesota R 595 by the phenol/chloroform/petroleum ether method (9,10). Zymosan was purchased from Sigma Chemical Co., St. Louis, MO. [^{3}H]-PGE_2, [^{3}H]-$PGF_2\alpha$ and [^{3}H]-6-keto-$PGF_1\alpha$ were purchased from New England Nuclear, Boston,1 MA. Anti-$PGF_2\alpha$ antiserum was prepared according to the method of Pesker et al.(11). Cross-reactivity of the antiserum with PGE_2 was 0.4 %. Antisera against PGE_2 were obtained from Sigma Chemical Co. St. Louis, MO.

Animals: Chinchilla-Bastard (Max-Planck-Institut für Immunbiologie) or Gelb-Silber (Borstel breed) rabbits, and 6 to 8-week-old female NMRI mice (Hannover) were used.

Cell preparations : Each rabbit was sacrificed by intravenous injection of 10 ml of air. The lung was then lavaged by inserting the oral cannula connected to the syringe into the trachea and flushing 20 ml of sterile saline into the lung. The procedure was repeated 4-5 times and the cells were harvested. About 1-2 x 10^8 cells were obtained from each rabbit. Mouse peritoneal cells were obtained by washing the peritoneal cavity with 5 ml of Iscove medium containing L-glutamine and 25 mM Hepes buffer, Gibco Lab. NY. These macrophages were allowed to adhere on the Tissue Culture Cluster 24 (Cambridge, MA) for 3 h at 37°C. After washing the cells three times with PBS (37°C), 1 ml of Iscove medium was added to each well and used for the tests.

Induction of prostaglandin release and its determination: Macrophages were incubated with the stimulant for 24 h at 37°C with 7.5% CO_2 in a humid atmosphere, the cells centrifuged and the prostaglandins in the supernatant were determined by radioimmunoassay (12).

Extraction and separation of prostaglandins: Prostaglandins released in the supernatant (1 ml of Iscove medium) were extracted with 1 ml of ethyl acetate twice after adjusting the pH to 3.0 with 1N HCl. The ethyl acetate was evaporated by blowing with nitrogen gas and the remaining prostaglandins were dissolved with 20 µl of methanol. Samples were then analyzed by high performance liquid chromatography (HPLC)(Gilson, model 802, pump model 302) using a column of RP-18 containing Lichrosorb. Elution was accomplished with a mixture solution of acetonitrile and water (32:68) containing 0.05% of acetic acid at a flow rate of 1.5 ml/min. The fractions corresponding to $PGF_2\alpha$ were collected and evaporated. The remaining $PGF_2\alpha$ was dissolved in 1 ml of Iscove medium and served for radioimmunoassay.

RESULTS

Dose dependency of endotoxin-induced prostaglandin release from rabbit alveolar macrophages. Prostaglandins released into the medium were estimated in the 5 x 10^5 cells/ml/well of rabbit alveolar macrophages treated with different concentrations of lipid A. As shown in Fig.1, the cells were found to secrete PGE_2 and $PGF_2\alpha$ even with 1 ng/ml of lipid A. Maximal prostaglandin production, 2.7 x 10^5 (PGE_2) and 7.5 x 10^4 pg/ml ($PGF_2\alpha$) was observed at a concentration of 0.3 µg/ml of lipid A. At higher doses the production of prostaglandins decreased probably because of the cell toxicity of lipid A. With the stimulation of LPS the cells also produced

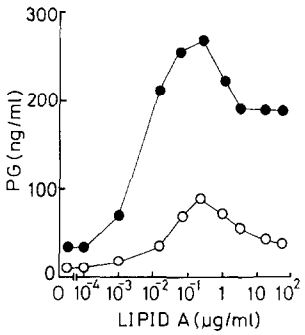

Fig.1. Dose dependency of lipid A-induced prostaglandin release from rabbit alveolar macrophages. 5 x 10^5 cells were cultured with different amounts of lipid A for 24h. Prostaglandins released were measured by radioimmunoassay. ●, PGE_2; ○, $PGF_{2\alpha}$.

prostaglandins. Maximal prostaglandin production (PGE_2: 7.2 x 10^4 pg/ml and $PGF_{2\alpha}$: 4.5 x 10^4 pg/ml) was however, observed at the highest LPS dose tested (100 μg).

The effect of prostaglandins on the LPS-induced prostaglandin release from alveolar macrophages. The effect of PGE_2 on the production of $PGF_{2\alpha}$ from macrophages stimulated with LPS was examined. Each of 0.03, 0.3, and 3 nmol of PGE_2 was incubated with 1 x 10^5 cells/ml/well in the presence or absence of different concentrations of LPS (0.1, 1, 10, 100 μg/ml) and the $PGF_{2\alpha}$ released into the medium was estimated after 24-h incubation. Identical concentrations of PGE_2 added in the medium without macrophages were used as a control, in order to negate any false value caused by cross-reaction in radioimmunoassay. When PGE_2 was added to the culture together with a stimulation dose of LPS (1 μg to 100 μg/ml), the production of $PGF_{2\alpha}$ was augmented greatly compared to that by LPS alone. The effect of PGE_2 was observed in all combinations of doses of PGE_2 and stimulating doses of LPS tested (Fig.2). On the other hand, no concentration of PGE_2 exhibited the induction of $PGF_{2\alpha}$ release when there was no LPS or when a nonstimulating dose of LPS (0.1 μg/ml) was used. In addition no effect was demonstrated by exogenous $PGF_{2\alpha}$ on the LPS-induced PGE_2 synthesis (data not shown). In order to ensure that the $PGF_{2\alpha}$ values estimated were not erroneous as a result of cross-reaction with PGE_2 added exogenously, supernatants of the test samples were extracted with ethyl acetate, applied to high performance liquid chromatography (HPLC), and the $PGF_{2\alpha}$ fraction was collected and estimated by radioimmunoassay. The results were

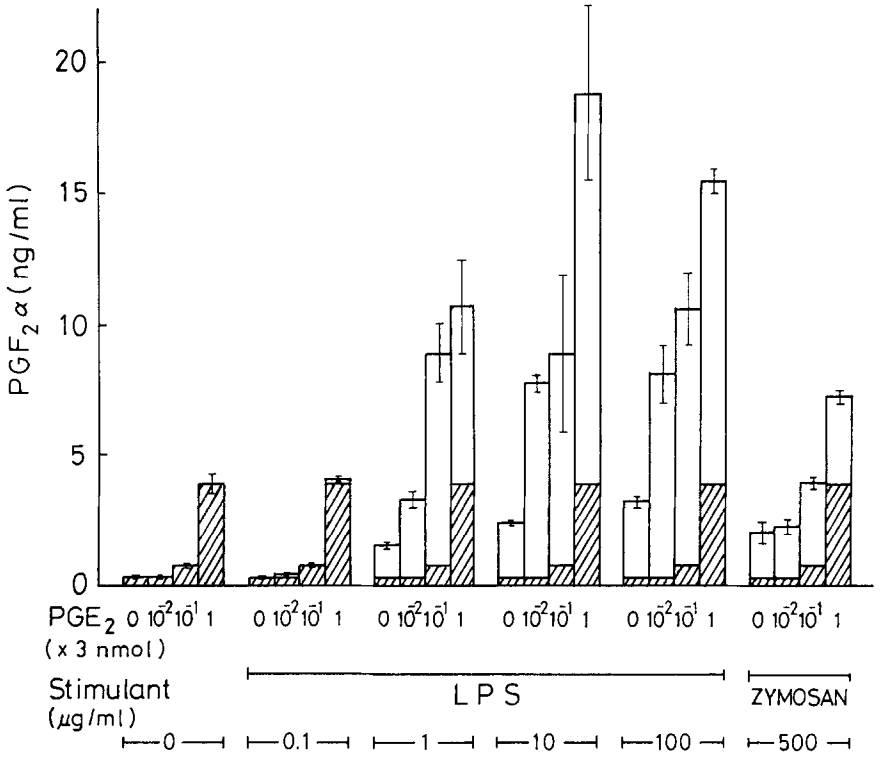

Fig.2. The effect of PGE_2 on the LPS- or zymosan-induced prostaglandin release in rabbit alveolar macrophages. 1 x 10^5 cells were incubated with different amounts of PGE_2 and LPS, or zymosan in the indicated combinations. Controls contained no LPS. After 24-h incubation the $PGF_2\alpha$ released into the medium was measured. Values are expressed as the mean ± SD. The cross-hatched area represents the false reading of $PGF_2\alpha$ caused by the cross-reaction by exogenous PGE_2 added initially. The area not cross-hatched represents the real $PGF_2\alpha$ released from macrophages.

consistent with those expressed as the unshaded area in Fig.2. Furthermore, the PGE_2 content of the $PGF_2\alpha$ fractions was no more than 200 pg in any sample. These results strongly support that the enhancement of LPS-induced $PGF_2\alpha$ release by PGE_2 was not caused by cross-reaction with exogenous PGE_2. Similar experiments were performed using PGI_2 instead of PGE_2. The sensitization by PGI_2 of the macrophages to LPS was more pronounced. $PGF_2\alpha$ induced by 10 or 100 μg/ml of LPS under the coexistence of 3 nmol of PGI_2 was 20.5 and 19.1 ng, respectively. When 500 μg of zymosan was used as a stimulant instead of LPS along with the existence of various concentrations of PGE_2, no significant effect was observed on the production of $PGF_2\alpha$ (Fig.3).

Time dependency of sensitization of alveolar macrophages by PGE_2 to the LPS-induced $PGF_2\alpha$ release. Ten micrograms of LPS were added to the culture at time 0. PGE_2 at different concentrations was administered at times -3, 0, 1, 3, 8 and 18 h and the

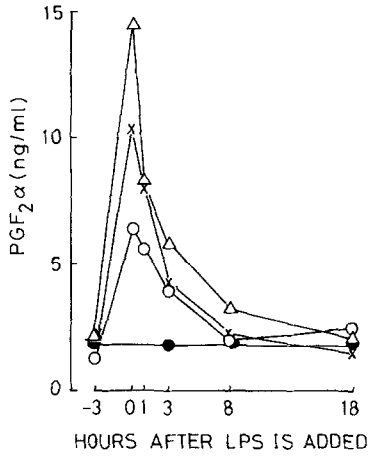

Fig.3. Time dependency of the enhancement of PGE_2 on the LPS-induced prostaglandin release in rabbit alveolar macrophages. The 1 x 10^5 cells were stimulated with 10 μg of LPS which was added to the culture at time 0. PGE_2 at different doses was administered at times -3,0,1,3,8,and 18 h. The amount of PGE_2 added : 3 nmol (Δ), 0.3 nmol (X), 0.03 nmol (○) and 0 (●). The culture was stopped at 24 h and $PGF_2\alpha$ released was estimated by radioimmunoassay. The values are expressed as amounts of $PGF_2\alpha$ corrected by subtracting the cross-reacted portion caused by the PGE_2 added initially.

time course of sensitization of the cells was examined. The effect of PGE_2 was most apparently expressed when it was administered to the culture at the same time as LPS. The effect decreased depending on the time elapsed since the addition of PGE_2 and after 8 h, no effect was observed. In addition, pretreatment of macrophages with PGE_2 3 h prior to the stimulation with LPS was not effective (Fig.3).

DISCUSSION

It has been shown that LPS can stimulate polymorphonuclear leukocytes or mouse peritoneal macrophages for the production of prostaglandins (7,13-16). The present study shows that alveolar macrophages also produce prostaglandins with stimulation by LPS or lipid A in a very augmented manner compared to mouse peritoneal macrophages.

During studies of the regulation of LPS-induced prostaglandin synthesis using rabbit alveolar macrophages, we found that certain prostaglandins enhance the sensitivity of the cells to LPS. Since exogenous PGE_1 or PGE_2 acts usually as the inhibitor of various macrophage functions (1-3), it was expected that PGE_2 acts as a feedback regulator of macrophage activation as was demonstrated by Schneider et

al.(17). Contrary to expectations, PGE_2 caused extraordinary enhancement of the activity of LPS for the induction of prostaglandin synthesis. The effect of PGE_2 was observed neither when it was added to the culture alone nor with a nonstimulating dose of LPS. The sensitizing activity of PGE_2 was even more pronounced when PGI_2 was used instead of PGE_2. The effect was, however, observed in rather restricted conditions, thus, i) the sensitization of macrophages by prostaglandins was not demonstrated on the cells stimulated with phagocytic stimuli (zymosan) (Fig.2). ii) The effect was not demonstrable by $PGF_{2\alpha}$ when used instead of PGE_2. iii) The phenomenon was observed in rabbit alveolar macrophages, but not in mouse peritoneal macrophages in our preliminary experiments. iv) The effect was expressed in the limited time range of PGE_2 addition; Simultaneous addition of PGE_2 with LPS was most effective and the addition of PGE_2 prior to the LPS or the addition 8 h after LPS had no effect (Fig.3). These facts suggest that PGE_2 is involved in the initial events of macrophage activation by LPS that lead to synthesis of $PGF_{2\alpha}$. A similar effect of PGE_2 has been found only by Wahl et al. using guinea pig peritoneal macrophages (4) who demonstrated that the simultaneous addition of PGE_1 or PGE_2 and endotoxin, elevated collagenase activity by 2 to 10-fold, although the addition of prostaglandins alone to macrophages did not stimulate collagenase production. In this study the sensitization of macrophages by exogenous PGE_2 was found to be manifested at a concentration of PGE_2 producible from macrophages stimulated with LPS. These findings indicate that certain prostaglandins can activate the cyclooxygenase pathway of arachidonic acid of macrophages in a LPS-stimulated state.

REFERENCES

1. Cantarow W.D., Cheung H.T. and Sundharadas G.(1978) Prostaglandins **16**, 39-46.
2. Oropeza-Rendon,R.L., Speth V., Hiller G., Weber K. and Fisher H. (1979) Exp. Cell. Res. **119**, 365-371.
3. Vassalli J.D., Hamilton J. and Reich E. (1976) Cell **8**, 271- 281.
4. Wahl L.M., Olson C.E., Sandleberg A.L. and Mergenhagen S.E. (1977) Proc. Natl. Acad. Sci. U.S.A. **74**, 4955-4958.
5. Erdoe E.G., Hinshow L.B.and Gill C.C. (1967) Proc. Soc. Exp. Med. **125**,916-919.
6. Skarnes R.C. and Harper M.J.K. (1972) Prostaglandins **1**, 191- 203.
7. Rietschel E.Th., Shade U., Lüderitz O., Fisher H. and Pesker B.A.(1980) Am. Soc. Microbiol., Washington D.C. 66-72.
8. Rietschel E.Th., Shade U., Jensen M., Wollenweber H-W., Lüderitz O. and Greisman S.G. (1982) Scand. J. Infect. Dis. (Suppl) 8-21.
9. Galanos C., Lüderitz O. and Westphal O.(1969) Eur. J. Biochem. **9**, 245-249.

10. Galanos C., Lüderitz O.and Westphal O. (1971) Eur. J. Biochem. **24**, 116-122.
11. Pesker B.A., Anhut H., Kroner E.E. and Pesker B.M. (1979) in Tillement J.P.(ed.) Advances in pharmacology and therapeutics, vol.7, Biochemical clinical pharmacology. Pergamon press, Oxford, New York, pp.341-348.
12. Jobke A., Pesker B.A. and Pesker B.M. (1973) FEBS Lett. **37**, 192-196.
13. Bray M.A.. Gordon D. and Morley J. (1974) Br. J. Pharmacol. **52**, 453
14. Higgs G.A. and Youlton L.J.F. (1972) Br. J. Pharmacol. **44**, 330
15. Kurland J.I. and Bockman R. (1978) J. Exp. Med. **147**, 952-957.
16. Wahl L.M., Rosenstreich D.L., Glode L.M., Sanberg A.L.and Mergenhagen S.E.(1979) Infect. Immun. **23**, 8-13.
17. Scyneider J., Dcwald B. and Baggiolini M. (1981) Prostaglandins **22**, 411-421.