Prostaglandin Biosynthesis in Pulmonary Macrophages

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Cultured rabbit alveolar macrophages, prelabeled with ^{14}C-arachidonic acid (AA), released into the medium a trace amount of labeled prostaglandins (PG) as well as their precursor, AA. Phagocytosis of zymosan, heat-killed Staphylococcus, or bacille Calmette-Guérin (BCG) increased the AA and PG release to 2–2.5 times control values. The released PGs consisted of PGE_2, D_2, $F_{2\alpha}$, and 6-keto $F_{1\alpha}$. Phagocytosis of latex particles had no effect on PG release. Indomethacin inhibited release of PGs but did not affect AA release at low doses. Analysis of the cellular lipids showed that zymosan decreased the radioactive label in phosphatidylcholine (PC), but not in other phospholipids or neutral lipids, suggesting that PC is the main source of AA for PG synthesis in pulmonary macrophages. Cytochalasin B (CB) at phagocytosis-inhibiting doses or below, markedly increased PG synthesis by zymosan-treated macrophages. These data suggest that PG release is not dependent on engulfment of the particles. Phagocytosis of zymosan (but not latex) also resulted in the release of two lysosomal enzymes, acid phosphatase and β-glucuronidase, which appeared temporally associated with the release of PGs (but not to phagocytosis). Furthermore, CB augmented the zymosan-stimulated release of these enzymes at the same doses stimulating PG synthesis. However, indomethacin, at a dose completely inhibiting PG synthesis, failed to block lysosomal enzyme release. Thus, the coincidental release of PGs and lysosomal enzymes is not the result of a regulatory role of PGs in the release of lysosomal enzymes, but probably is the result of a common pathway of stimulation. (Am J Pathol 97:137–148, 1979)

ALVEOLAR MACROPHAGES play a central role in pulmonary inflammatory responses. Their role in sequestering and destroying infectious agents and removing noxious particles is well known. Recently, there has been interest in the secretory functions of pulmonary macrophages.[1] Macrophages secrete material that influences granulocytopoiesis,[2] B- and T-lymphocyte function,[3] fibroblast proliferation,[4,5] and angiogenesis.[6] Recent studies have suggested that prostaglandins (PGs) are among the mediators influencing the interactions between macrophages and other cells.

It has been known for several years that polymorphonuclear leukocytes produce PGs [7–10] and possibly thromboxanes [11] during phagocytosis. It was not until recently that attention was directed to the role of macrophages

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in PG production. PGE-like material was demonstrated by radioimmunoassay in macrophage-rich peritoneal exudate cell preparations from guinea pigs [12-14] and mice.[15,16] Furthermore, PGE_2, 6-keto-$PGF_{1\alpha}$, and $PGF_{2\alpha}$ have been observed to be released by mouse peritoneal macrophages in response to inflammatory stimuli such as zymosan and *Corynebacterium parvum*.[15,16,17]

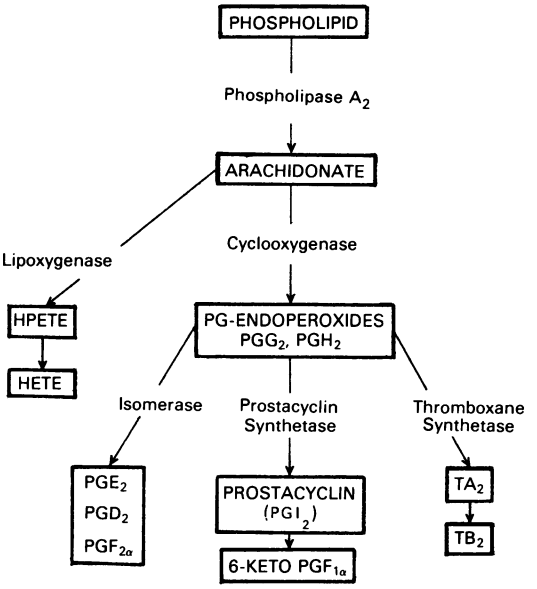
The role of lysosomal enzymes as mediators of inflammatory reaction is well documented.[18-20] Endocytic stimuli have been shown to result in increased lysosomal enzyme activity in mouse peritoneal macrophages [18,21,22] and polymorphonuclear leukocytes (PMN).[18,22] However, the mechanisms for lysosomal enzyme induction are poorly understood. Since PGs are also mediators of inflammation [23,24] and are secreted in response to phagocytic stimuli, it is important to examine the relationship between PG production and lysosomal-enzyme release in phagocytosis.

All macrophages are presumably derived from circulating blood monocytes.[25] During their maturation in peripheral tissues they acquire specialized properties. Differences have been reported between pulmonary and peritoneal macrophages in several respects, including reaction to antimacrophage serums,[26,27] metabolism,[28,29] ultrastructure,[30] and regulation of protease secretion.[31] All previous work on PG production by macrophages was done using peritoneal macrophages. In view of the apparent structural and functional differences, the study of PG synthesis in pulmonary macrophages seems warranted.

The principal objectives of the present study are to identify the various PGs secreted by alveolar macrophages and to examine their relationship to phagocytosis and lysosomal enzyme release.

Materials and Methods

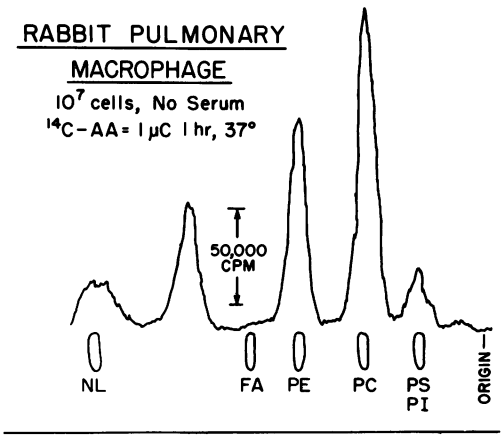
Prostaglandins are not stored in cells but are released as soon as they are formed. Their immediate precursor is arachidonic acid (AA). Free fatty acid levels are very low in cells,[34] and the synthesis and release of PGs requires the activation of cell lipase to release arachidonic acid from bound tissue lipids, predominantly phospholipids.[35,36] The released arachidonic acid is then converted by the enzyme cyclooxygenase to the unstable endoperoxide intermediates PGG_2 and PGH_2,[37] which in turn are metabolized to several PG end-products with varying biologic activities (Text-figure 1). We have previously developed methods of incorporating ^{14}C-arachidonic acid into the tissue lipid pool in both isolated organs [38] and tissue culture,[39] and of measuring the free arachidonic acid release by using the fatty-acid-free bovine serum albumin to "trap" the released free fatty acid and prevent its use for reacylation.[38,40] By labeling the precursor pool in the cell and by measuring the subsequent release of radioactive arachidonic acid and PGs, we could quantitatively determine both the lipase and cyclooxygenase activities in the cells following the use of various stimuli.



TEXT-FIGURE 1—Biosynthesis of the primary prostaglandins and thromboxanes from anachidonic acid.

Results

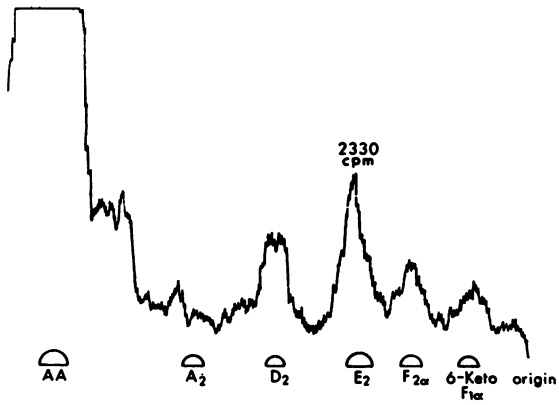
When alveolar macrophages, obtained by bronchopulmonary lavage, were cultured overnight, washed, and incubated 90 minutes with labeled ^{14}C-arachidonate (specific activity 55 mCi/mM, 1–2 × 10^6 dpm/ml) for 1 hour, approximately 40% of the radioactivity was incorporated into cell lipids. Approximately two-thirds of the radioactivity was incorporated into phospholipids, predominantly into phosphatidylcholine and phosphatidyl ethanolamine (Text-figure 2). Only 10% of the label was incorporated into neutral lipid, and less than 2% of the label in the cells was present as free fatty acid.[41] Rabbit pulmonary macrophages allowed to phagocytize zymosan (500 μg/ml) released a mixture of PGE_2, D_2, $F_{2\alpha}$, and 6-keto-$F_{1\alpha}$ (the stable metabolite of PGI_2). Typical radiochemical scans of extracts prepared from the culture medium following zymosan stimulation of rabbit pulmonary macrophages are shown in Text-figure 3. Resting alveolar macrophages only release trace amounts of PGs. Phagocytosis of zymosan increased the release of PGs and arachidonic acid to 2.5 times the control values. Ingestion of heat-killed *Staphylococcus aureus* and bacelle Calmette-Guérin (BCG) elicited a similar response. Indomethacin inhibited the release of PGs, but not of arachidonic acid. However, ingestion of inert latex particles (1.1 μ) had no effect on PG or arachidonic acid release (Text-figure 4). The proportion of different PGs varied with the



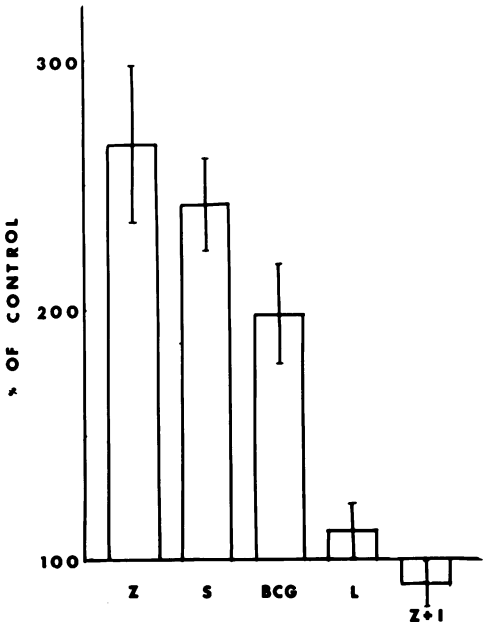
TEXT-FIGURE 2—Incorporation of ^{14}C-arachidonic acid into cell lipid. The cells were extracted with 20 volumes of chloroform : methanol (2 : 1) at the end of the experimental period (90 minutes), applied on thin-layer plates, and developed in chloroform : methanol : ammonia (65 : 35 : 5). *PC* = phosphatidylcholine; *PE* = phosphatidylethanolamine; *PS* = phosphatidylserine; *PI*=phosphatidylinositol; *FA* = fatty acid; and *NL* = neutral lipids.

concentration of albumin in the medium. In the presence of 1 mg/ml (or more) bovine serum albumin there was a significant increase of PGD_2 (from 14% of the total PGs to 24%) with a corresponding decrease of PGE_2 and $F_{2\alpha}$, as shown in Table 1. These data suggest the possibility of secretion of PG endoperoxide into the mediums, since bovine serum albumin has been reported to facilitate the conversion of endoperoxide to PGD_2.[43]

With the above-mentioned radiochemical techniques no thromboxanes could be detected in either resting or activated macrophages. However, incubation of cell homogenates with ^{14}C-PGH_2 lead to a small yield of thromboxane: 7–8% of the radioactivity was detected in the zone corresponding to the thromboxane B_2 standard. This thromboxane peak could be inhibited by preincubation for 5 minutes with imidazole (200 μg/ml), an inhibitor of thromboxane synthetase.[44] These data indicate the pres-



TEXT-FIGURE 3—Radiochemical scans of extract of medium from a typical experiment showing release of ^{14}C-PGs following zymosan stimulation (90 minutes). The medium was collected, extracted, applied on thin-layer plates, and developed in solvent system A-9.[42]



TEXT-FIGURE 4—Change in total ^{14}C-PG production by cultured alveolar macrophages in response to various stimuli (mean ± SEM). The mediums were extracted at the end of experimental period (90 minutes), applied on thin-layer plates, and developed in solvent system A-9. Zones corresponding to various PG standards were scraped and counted. The total PG release (sum of 6-keto-$PGF_{1\alpha}$ + $PGF_{2\alpha}$ + PGE_2 + PGD_2) was calculated and expressed as the percentage of the control value (unstimulated cells). Z = zymosan (1 mg/ml); *S* = heat-killed *Staphylococcus aureus* (10^9 cells); *BCG* = heat-killed 1 mg bacille Calmette-Guérin; *L* = latex particles (1.1 μ); *I* = indomethacin (20 μg/ml). Data are expressed as the mean ± SEM of three experiments.

ence of thromboxane synthetase in alveolar macrophages. Cytochalasin B was used to dissect different stages of phagocytosis, since this drug inhibits the internalization of particles but does not affect the attachment of the particle to the macrophage membrane.[32,33]

The addition of a low dose of cytochalasin B (0.5 μg/ml) at the same time as the zymosan to macrophages resulted in a further increase of PG release to 3.4 times control values[41] without changing the proportion of various PGs. A parallel increase in arachidonic release (6.6 times the con-

Table 1—The Effect of Bovine Serum Albumin (BSA) in the Mediums on Zymosan-Induced Prostaglandin (PG) Production by Alveolar Macrophages

BSA	Total ^{14}C-PG (cpm)	6-Keto-$PGF_{1\alpha}$ (%)	$PGF_{2\alpha}$ (%)	PGE_2 (%)	PGD_2 (%)	^{14}C-AA: ^{14}C-PG ratio
Present (n = 10)	4221 ± 731	18 ± 1	23 ± 1	39 ± 3	24 ± 1	8 ± 1
Absent (n = 6)	4338 ± 1016	19 ± 1	25 ± 1	44 ± 2	14 ± 1	0.3 ± 0.1

Ninety minutes after addition of zymosan, the mediums were collected, extracted, applied to thin layer plates, and developed in solvent system A-9.[42] Zones corresponding to various PG standards and arachidonic acid were scraped and counted. PGs are expressed as the percentage of total PGs released (sum of 6-keto-$F_{1\alpha}$ + $F_{2\alpha}$ + E_2 + D_2).

trol value) indicates that cytochalasin B increased PG release by activating cell lipases. Inhibition of phagocytosis was not demonstrated at this dose. The addition of cytochalasin B at doses of 2 μg/ml or more showed 30% inhibition of phagocytosis of zymosan. However, despite the decrease of particle engulfment, the release of PGs and arachidonic were further enhanced [41] to 4.3 times, and 9 times, respectively. Cytochalasin B alone, without zymosan, had no effect on the release of PGs and arachidonic acid; neither did cytochalasin B with latex.

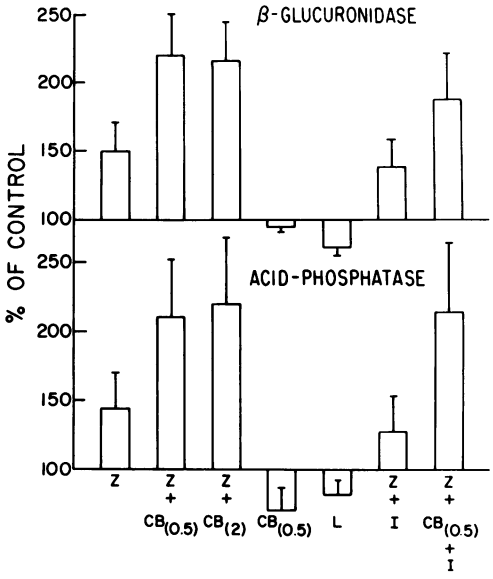
Analysis of the cell lipids shows a decrease in the fraction of label in phosphatidylcholine by 4% of the total labeled lipid after zymosan stimulation. The decrease was even greater (7% ± 0.7%) after the addition of cytochalasin B with zymosan. This loss of radioactivity from phosphatidylcholine was roughly equivalent to the molar amount of PGs and arachidonic acid released into the mediums. No decrease of labeled phosphatidylethanolamine or neutral lipid could be detected. Thus, the main source of arachidonic acid for PG synthesis in alveolar macrophages appears to be phosphatidylcholine.

Resting macrophages secreted less than 10% of their total lysosomal enzymes β-glucuronidase and acid phosphatase during a 90-minute incubation. Phagocytosis of zymosan by macrophages resulted in a 50% increase of both β-glucuronidase and acid-phosphatase release [41] (Text-figure 5). This increase was further augmented to three times the control value by the addition of cytochalasin B (0.5 μg/ml) with the zymosan. A correponding decrease in intracellar lysosomal-enzyme content was observed in the cells treated with zymosan or zymosan with cytochalasin B. Neither cytochalasin B alone nor latex affected lysosomal enzyme release. Indomethacin at doses completely inhibitory to PG synthesis (2 μg/ml) did not significantly change the levels of these enzymes.

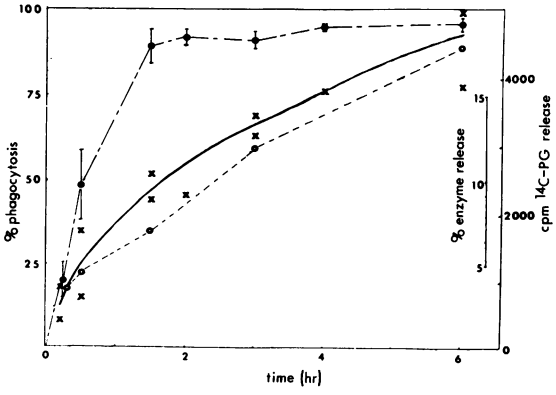
Study of the time course of phagocytosis, lysosomal enzyme release, and PG synthesis revealed that the release of PG temporally paralleled the release of lysosomal enzyme, but not phagocytosis (Text-figure 6).

Discussion

It has been recently shown that PMN leukocytes and mouse peritoneal macrophages produce PGs during phagocytosis.[7-17] The present study shows that rabbit alveolar macrophages also produce PGs, including PGE_2, D_2, $F_{2\alpha}$, and I_2. Thromboxane synthetase probably exists in the cells, as demonstrated by the production of thromboxanes by incubating PGH_2 directly with cell homogenates. However, the number of thrombo-



TEXT-FIGURE 5—Change in lysosomal enzyme release following various stimuli in rabbit alveolar macrophages. The mediums were collected after 90 minutes incubation, and aliquots were used for lysosomal enzyme determination. Both β-glucuronidase and acid phosphatase were determined fluorimetrically.[45] Data are expressed as the mean ± SEM of three experiments. Upper, β-glucuronidase; lower, acid phosphatase. Z = zymosan (1 mg/ml); $CB_{0.5}$ = cytochalasin B (0.5 μg/ml); CB_2 = cytochalasin B (2 μg/ml); *I* = indomethacin (2 μg/ml); *L* = latex particles (0.1 mg/ml).



TEXT-FIGURE 6—Time course of phagocytosis of zymosan, lysosomal enzyme release, and PG production by alveolar macrophages. Zymosan was added at time zero, and mediums were collected at different time intervals for analysis of acid phosphatase activity and PG production. The cells were fixed with 70% alcohol and stained with periodic acid–Schiff (PAS) reaction, and the percentage of cells containing two more zymosan particles (magenta color by PAS stain) was recorded. The acid-phosphatase activity of the medium and the cells was determined by a fluorometric method,[45] and the enzyme release was expressed as percentage of intracellular enzymes released into the medium. The PG production was determined by radiochemical techniques as described above. X —— X, ^{14}C-PG production; O ---- O, percentage of intracellular acid phosphatase released into the medium; ●—·—●, percentage of cells containing phagocyted zymosan particles.

Table 2—Thromboxane Synthesis by Cell Homogenates of Rabbit Alveolar Macrophages

Treatment applied	Total PGs (cpm)	6-Keto-$PGF_{1\alpha}$ (%)	$PGF_{2\alpha}$ (%)	PGE_2 (%)	PGD_2 (%)	TB_2 (%)
No treatment (n = 4)	15152 ± 1552	7 ± 1	31 ± 3	40 ± 2	23 ± 1	8 ± 1
Imidazole* (n = 3)	14099 ± 522		29 ± 1	40 ± 1	26 ± 1	3 ± 0
Boiling water† 5 min (n = 3)	14072 ± 1117	3 ± 1	28 ± 1	39 ± 1	28 ± 1	3 ± 0.4

Alveolar macrophage homogenates were incubated with 5×10^4 cpm ^{14}C-PGH_2 at 37C, shaking water bath for 15 minutes. The mediums were immediately acidified and extracted with ethyl acetate and were divided into two equal portions. One was applied on thin-layer plates and developed in system BDA (benzene: diozane: acetic acid, 60:30:3) to separate thromboxanes from the prostaglandins. The other was plated and developed in system A-9.[42]

* The cell homogenates were preincubated with imidazole (200 μg/ml) for 5 minutes before the addition of PGH_2.

† The cell homogenates were placed in boiling water for 5 minutes before incubation with PGH_2. Note the high $PGF_{2\alpha}$, PGD_2, and PGE_2 of this group, which were not significantly different from the other two groups. These data indicate that the production of these PGs (PGE_2, D_2, and $F_{2\alpha}$) was largely due to the spontaneous degradation of the endoperoxide PGH_2.

xanes produced by the intact macrophages was too small to be detected by the radiochemical techniques applied. Inflammatory stimuli, such as zymosan, BCG, and *Staphylococcus aureus* brought about PG release by activation of cell lipases. In contrast, inert particles, such as latex, were not stimulatory. This suggests that PGs may play an important role in response to bacterial infection and other inflammatory processes *in vivo*.

Cytochalasin B, whether at doses inhibitory or noninhibitory to phagocytosis, enhanced PG synthesis when added to zymosan-treated cells. This result suggests that stimulation of PG synthesis by zymosan treatment was not dependent on the uptake of the particles.

The observations reported here raise the possibility of a relationship between lysosomal enzyme release and PG secretion. Both processes were evoked by the "inflammatory" stimulus, zymosan, but not by the "inert" stimulus, latex particles. Both PG release and lysosomal enzyme release were enhanced by the addition of cytochalasin B to zymosan-treated cells, whereas cytochalasin B alone had no effect. Furthermore, PG synthesis and lysosomal enzyme release followed a similar time course, following phagocytosis, after a short lag. One explanation of the parallelism between PG secretion and lysosomal enzyme release might be that PGs regulate lysosomal enzyme secretion. This possibility has been excluded, since indomethacin, at doses that completely inhibited PG synthesis, failed to inhibit lysosomal enzyme release. It may be that some common effector stimulates both PG synthesis and lysosomal enzyme release.

Recent investigations have shown PGs to be among the mediators between macrophages and other cells. For instance, PGs have been observed to depress myelopoiesis induced by colony-stimulating factor [46]; to depress lymphokine production [47]; to depress the plaque-forming response to Hb autologous erythrocytic antigen [48]; to prevent macrophage enhancement of B-lymphocyte colony formation [49]; and to depress the mitogenic response of T lymphocytes in Hodgkin's disease.[50]

The present work shows that inflammatory materials stimulate production of a variety of prostaglandins from alveolar macrophages. These prostaglandins may be important in modulating the cellular interactions that together make up the inflammatory response in the alveoli.

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