# PROSTAGLANDIN AND THROMBOXANE SYNTHESIS IN A PURE MACROPHAGE POPULATION AND THE INHIBITION, BY E-TYPE PROSTAGLANDINS, OF CHEMILUMINESCENCE

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## 1. Introduction

Cell populations consisting predominantly of macrophages emit chemiluminescence when they are phagocytosing zymosan particles [1,2] or bacteria [1,3,4]. This chemiluminescence is considered indicative of the generation of reactive species of oxygen ($O_2^-$, $H_2O_2$, $OH^\cdot$), which are formed as intermediates during the stepwise reduction of molecular oxygen by one-electron donors, and of singlet $O_2$ ($^1\Delta O_2$) generated during the disproportionation of $O_2^-$. These species emit light in the presence of polyunsaturated fatty acids, polysaccharides or easily oxidizable substances like luminol [5]. As these species of oxygen are considered important in the killing mechanism of phagocytic cells [6] chemiluminescence may be viewed as a manifestation of their microbicidal activity [7]. Independently it has been reported that macrophages form and release prostaglandins (PGs) [8] under conditions comparable to those that evoke chemiluminescence. PGs have been implicated as mediators in inflammation and cellular immunity [9–11].

We have studied chemiluminescence, PG formation and their possible interrelationship in macrophages cultivated from bone marrow precursor cells of mice. By morphological criteria these cells are not contaminated by granulocytes or lymphocytes and appear as a pure population of macrophages [12].

During phagocytosis of zymosan these cells produce chemiluminescence and immunoreactive PGs mainly of the E-type, but also measurable amounts of $PGF_{2\alpha}$. In addition, we have found significant amounts of thromboxane $B_2$($TXB_2$). Chemiluminescence, PG and TX synthesis can also be elicited in the absence of phagocytosis by the bivalent metal ion ionophore A23187. Most important, however, the chemiluminescence in either case can be inhibited by PGs of the E-type. The latter findings suggest that PGs released by macrophages may, apart from other immune cells [9–11, 13–16], also control the activity of the macrophage itself.

## 2. Materials and methods

Zymosan, lot no. 6757, was obtained from ICN Pharmaceuticals, Cleveland, OH. The bivalent metal ion ionophore A23187 [17] was kindly provided by Dr R. L. Hamill (Eli Lilly). All PGs and $TXB_2$ were gifts from Dr J. Pike (Upjohn Co., Kalamazoo). Macrophages were cultivated from bone marrow cells obtained from C57/B1 mice and were harvested on days 6–8 [12]. Chemiluminescence was measured at 37°C in a liquid scintillation spectrometer (Packard Tri-carb model 3002) set in the off-coincidence mode. PGs and $TXB_2$ were estimated by radioimmunoassay as in [18].

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## 3. Results

Addition of zymosan particles to a suspension of macrophages ($10^6$ cells/5.0 ml) led, after a delay of 10 min, to a significant increase in chemiluminescence that reached peak intensity in 50–70 min and thereafter declined (fig.1a). This response could be detected in the absence of luminol. Its intensity was not significantly altered if the cells were allowed to adhere to the walls of the glass vials. The adherent cells responded more quickly to zymosan, however, and the maximum chemiluminescence occurred only 20–30 min after its addition (results not shown). In each case mainly immunoreactive $PGE_2$, but also smaller amounts of $F_{2\alpha}$ as well as $TXB_2$ accumulated in the incubation medium during the period of stimulated chemiluminescence (fig.1b). The synthesis of PGs and $TXB_2$ was completely blocked by 28 μM indomethacin, which failed, however, to affect chemiluminescence under the experimental conditions used (table 1).

A similar, but greatly accelerated, chemiluminescence response was obtained in the absence of phagocytosis when macrophages were incubated with the ionophore A23187 at a predetermined optimal concentration (fig.2a). This response of less than 4 min duration, could be demonstrated only in the presence of luminol (5-amino-2,3-dihydro-1,4 phtalazinedione)

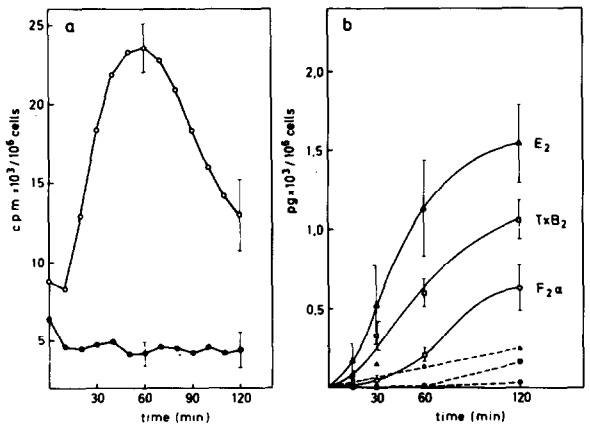


Fig.1. Time courses of chemiluminescence (a) and prostaglandin and thromboxane synthesis (b) in bone-marrow-derived cultured mouse macrophages after addition of zymosan. Freshly harvested macrophages ($10^6$) were suspended in 5.0 ml Eagle's medium buffered with 20 mM Hepes, pH 7.4; after temperature equilibration, the chemiluminescence was measured at 37°C in a Packard Tri-carb spectrometer. Zymosan (2.5 mg) was added and the light emission measured for 0.2 min intervals (in duplicate) every 10 min for 2 h (fig.1a (○-○)). At the end of the incubation period shown (fig.1b), the vials were cooled on ice, the cells sedimented (400 × *g*, 10 min) and PGs $F_{2\alpha}$ (○-○) and $E_2$ (△-△) as well as $TXB_2$ (□-□) present in the incubation medium were determined, without prior extraction. In each case, control incubations were carried out without the addition of zymosan (closed symbols in fig.1a,b). All values are corrected for the concentrations of PGs and $TXB_2$ present in the incubation medium. The results are expressed as means ± SEM of 4 (in fig.1a) and 6 (in fig.1b) experiments, respectively.

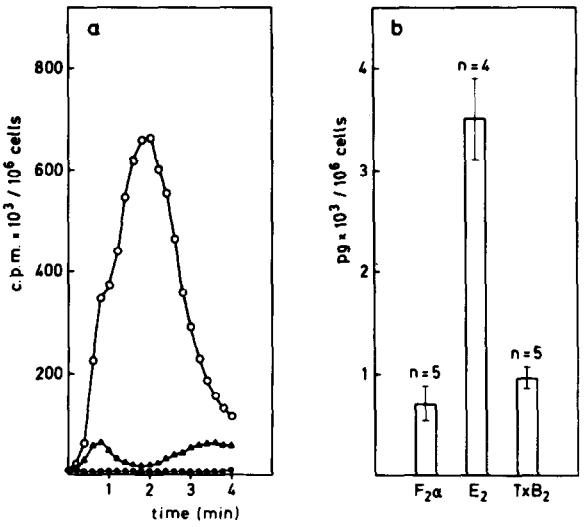


Fig.2. Effect of the ionophore A23187 on chemiluminescence (a) and prostaglandin and thromboxane synthesis (b) in bone-marrow-derived cultured mouse macrophages ($10^6$). Chemiluminescence was measured at 12 s intervals after the addition of 0.1 ml luminol-saturated foetal calf serum [3]: to cells only (●-●); to cells after addition of the optimum concentration, judged by peak chemiluminescence, of ionophore A23187 (0.5 μM) (○-○); to cells after 0.5 μM A23187 following a 1.0 min preincubation with 2.8 μM prostaglandin $E_1$ (△-△). Prostaglandins and thromboxane $B_2$ were measured in the incubation medium from which the cells had been removed by centrifugation (400 × *g*, 10 min) 15 min after the addition of 0.5 μM A23187. 5 μl dimethylsulphoxide or 5 μl 70% ethanol, the solvents used for A23187 and $PGE_1$, respectively, had no effect on chemiluminescence or prostaglandin synthesis. Other experimental conditions were as in fig.1, except that incubation medium was total vol. 2.5 ml. The results of a typical experiment are given in fig.2a; in fig.2b, the results are expressed as means ± SEM of A23187-stimulated cells from which control values (i.e., from cells incubated in solvent alone) have been subtracted.

Table 1
Effect of added prostaglandins and indomethacin on zymosan-dependent chemiluminescence in bone-marrow-derived mouse macrophages[a]

| Treatment | μM | Peak chemiluminescence as % control | | | |
|---|---|---|---|---|---|
| | | $\bar{X}$ | SE | $n$ | $P$ |
| Control | | 100 | – | 6 | – |
| Indomethacin in | 28 | 100.4 | 8.1 | 6 | n.s. |
| $PGE_1$ | 0.28 | 71.5 | 5.9 | 6 | <0.01 |
| $PGE_1$ | 2.80 | 61.2 | 5.4 | 6 | <0.005 |
| $PGE_2$ | 0.28 | 76.3 | 6.3 | 5 | <0.025 |
| $PGE_2$ | 2.80 | 71.0 | 5.8 | 5 | <0.02 |
| $PGF_{1\alpha}$ | 0.28 | 98.9 | 6.3 | 5 | n.s. |
| $PGF_{1\alpha}$ | 2.80 | 96.6 | 7.0 | 5 | n.s. |
| $PGF_{2\alpha}$ | 0.28 | 90.0 | 5.8 | 5 | n.s. |
| $PGF_{2\alpha}$ | 2.80 | 92.3 | 7.4 | 5 | n.s. |

[a] Experiments performed as in fig.1. The peak chemiluminescence of 6 control experiments was 16 643 ± 1667 cpm (mean ± SEM). Student's $t$-tests for paired observations were performed on the raw data with $\alpha > 0.05$ considered non-significant (n.s.). Prostaglandins were added in 5 μl 70% ethanol, which had no effect on chemiluminescence

[5]. This compound replaces in these experiments zymosan as an indicator for the presence of one or several of the reactive oxygen species mentioned above. A23187 also induced the synthesis of large amounts of $PGE_2$ as well as some $F_{2\alpha}$ and $TXB_2$ (fig.2b). Their formation was inhibited by indomethacin at low concentrations (0.1–1.0 μM) that had no significant effect on the accompanying chemiluminescence (results not shown).

PGs of the E-type, but not $PGF_{1\alpha}$ and $PGF_{2\alpha}$, significantly suppressed the chemiluminescence associated with zymosan addition (table 1), and addition of $PGE_1$ blocked the chemiluminescence induced by A23187 (fig.2a). Furthermore, a reversible inhibition of particle uptake, associated with striking changes in cell morphology, has also been observed in $PGE_1$-treated macrophages (R. L. Oropeza-Rendon, V. Speth and H. F., unpublished observations). The possibility that these inhibitory effects might extend to PG synthesis itself was not tested in the present work because of the interference of the added concentrations of $PGE_1$ with the radioimmunoassays of $PGE_2$, $PGF_{2\alpha}$ and $TXB_2$.

## 4. Discussion

The present finding that PG synthesis is inhibited by indomethacin, while chemiluminescence is not, indicates that these two processes are not firmly coupled in macrophages. The failure, on the other hand, to see chemiluminescence enhanced in the presence of indomethacin indicates that under our experimental conditions the endogenously produced concentrations of PGE are too low to affect chemiluminescence. The fact, however, that PG synthesis and chemiluminescence can be evoked by common stimuli suggests that these two processes may have at least one early step in common. This step, as suggested by the ionophore-induced stimulation of both processes, may relate to either an increase in the steady-state level of cytoplasmic $Ca^{2+}$ or to the specific activation of a $Ca^{2+}$-requiring enzyme. The activation of a $Ca^{2+}$-requiring phospholipase A [19], for example, which is known to be the rate-limiting reaction for PG synthesis [20,21] in a number of tissues, is an obligatory step under conditions where the precursor fatty acids are derived from endogenous

phospholipids [22]. The calcium ionophore A23187 has indeed been shown to activate phospholipase $A_2$ in platelets [23], and there is evidence that this also occurs in several other tissues, including lymphoma cells and polymorphonuclear leucocytes [24]. However, it is not clear at present, whether, or how, phospholipase activation is involved in the initiation of chemiluminescence.

Prostaglandins have been suggested as mediators between immune cells [9–11, 13–16], and E-type PGs have been shown to inhibit several lymphocyte functions, like production of lymphokines [10], cytolytic activity [25], or the response to mitogens [13,16]. The results presented here indicate that the PGs released by macrophages may also have a modulating effect on these cells themselves. Phagocytosis is inhibited by the E-type PGs, and the observed suppression in chemiluminescence may be taken as indication of an inhibition of the microbicidal activity of these cells [7]. The possible pathophysiological significance of these processes is emphasized by the fact that macrophages produce mainly PGs of the E-type, and only the E-types exert such effects. Furthermore, the PG concentrations used in these experiments (280 nM) are of the same order of magnitude as those found in some inflammatory conditions in vivo, e.g., in synovial fluids of patients with rheumatoid arthritis [26] or in epidermis of psoriasis [27].

An interesting feedback mechanism for the regulation of cellular immune response was proposed [10] and it was found that macrophage-born PGs of the E-type inhibit lymphokine secretion of lymphocytes. Our findings that PGs of macrophages could exert some kind of feedback control on the activities of these cells themselves adds a new aspect to macrophage–lymphocyte interaction in inflammatory and immune reactions.

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