Enhanced Activity of Murine Peritoneal Cells after Aclacinomycin Injection: Characteristics of the Enhanced Respiratory Burst

A. Bravo-Cuellar, F. Homo-Delarche, J. Cabannes, and S. Orbach-Arbouys

ABSTRACT

After the i.p. injection into normal mice, of 4 mg/kg of aclacinomycin (ACM), a dose which prolongs the survival of tumor-bearing mice, the zymosan-elicited chemoluminescence (CL) of the peritoneal cells (PC) is greater than that of control cells. The volume in which the drug is administered plays an important role in the intensity of the response. ACM also stimulated the CL of PC from tumor-bearing mice. It is known that CL can also be elicited by soluble stimuli such as 4α-phorbol-12-myristate-13-acetate or Ca²⁺ ionophore A 23187, which, however, act in different ways. The response of ACM cells to these stimuli is also greater than in control cells. The enhanced CL of ACM-treated cells can be inhibited by incubating in vitro the zymosan-triggered PC with superoxide dismutase (300 units/ml) and catalase (2750 units/ml), but not with ethanol (20 μM) or potassium cyanide (100 μM). This indicates the participation of O₂⁻ and H₂O₂ in the CL of ACM-treated cells, whereas mitochondrial respiration does not appear to be involved.

Furthermore, the following facts suggest the participation of arachidonic acid metabolism in the control of CL: (a) in vitro addition of nordihydroguaiaretic acid (7 x 10⁻⁵ M) and indomethacin (10⁻⁴ M) inhibits the CL, whereas indomethacin (10⁻⁴ M) has the opposite effect; (b) the PC from normal or ACM-treated mice when stimulated with zymosan secrete high amounts of prostaglandin (PG); (c) treated cells secrete the same amounts of PGE₂ and 6-keto-PGF₁α, but the secretion of PGF₂α, and particularly of thromboxane B₂, is greater in treated cells than in control cells and indomethacin (10⁻⁴ M) strongly inhibits PG secretion in all groups; (d) in vitro addition of PGE₂; at a concentration of 10⁻⁶ M has an inhibitory effect on the CL emission of control and of treated cells, but it does not have this effect at lower concentrations (10⁻⁶ M). These data suggest that the lipoxygenase pathway of arachidonic acid metabolism may be involved in the triggering of CL of ACM-treated cells, as well as that of normal cells, whereas products of the cyclooxygenase pathway may act as feedback inhibitors.

INTRODUCTION

Cancer-bearing patients are threatened by the continuous growth of their tumor, by infectious diseases, and by various side effects of the therapy to which they are submitted. Activated macrophages may help to control the former two points since they constitute an important defense mechanism against infectious agents and tumor cells (1, 2). Although in most cases chemotherapy acts as an immunosuppressive agent, recent reports have indicated that some antitumoral drugs may potentiate macrophage activity (3, 4).

This is the case for aclacinomycin, an antibiotic of the anthracycline family. We have observed that, after the i.p. injection of ACM² in mice, peritoneal cells have an increased antitumoral activity and emit more chemoluminescence when stimulated with zymosan than normal cells. This stimulatory effect of ACM is dose-dependent and can only be observed after i.p. administration. Injection of 4 mg/kg on day −4 leads to optimal CL stimulation. The macrophage is the stimulated cell among the peritoneal cell population (5).

The intensity of the CL emission by phagocytic cells is at least partly dependent on the release of reactive oxygen intermediates (6), but more recent reports (7–9) have described the role of other factors, such as those associated with arachidonic acid metabolism.

The present paper studies different conditions which modify the ACM-induced stimulation of PC chemoluminescence and indicates that the metabolic events of such stimulation are related to secretion of free oxygen radicals and to products of AA metabolism. Compared to control mice, PC from untreated tumor-bearing mice emit less CL. In contrast, in ACM-treated tumor-bearing mice, the CL is higher than that observed in normal cells, but its intensity is lower than that observed with normal ACM-treated cells. Our data also stress the importance of the mode of drug administration.

MATERIALS AND METHODS

Animals. Specific pathogen-free male C57BL/6 × DBA/2 F₁ mice, 6 weeks old, were obtained from the breeding center of the Institut de Recherches Scientifiques sur le Cancer (Villejuif, France). They were used within 2 weeks of delivery.

Preparation of Peritoneal Cells. Mice were sacrificed by cervical dislocation and peritoneal cells were obtained by washing the abdominal cavity with 6 ml of Hanks' solution without phenol red at 4°C (pH 7.2). Smears were routinely prepared by cytocentrifugation and colored with Giemsa and nonspecific esterase stainings for differential counts. Live cells were numbered by trypan blue exclusion and viability was >98%. In some experiments PC were purified by plastic adherence. The percentage of macrophages was estimated in Giemsa-stained smears before and after plastic adherence. For CL assay, 0.5 ml of a suspension containing 3 x 10⁶ macrophages was put in plastic cuvets (Clinicon). The cells were allowed to adhere for 2 h at 37°C in a 5% CO₂ atmosphere. The medium and the nonadherent cells were aspirated and the cuvets were washed three times with phenol red-free Hanks' balanced salt solution. At the end of each test the adherent cells were detached with EDTA at 4°C; approximately 80% of the adherent cells were found to be macrophages. In other experiments macrophages were completely removed by carbonyl iron phagocytosis (10) and 95% of the cells found to be lymphocytes.

Tumor Cells. Murine L1210 leukemic cells were maintained by weekly in vivo passages of 2 x 10⁶ cells in syngeneic mice. For the test, 1 x 10⁶ leukemic cells were injected s.c. into the left foot. Mice survived 18 ± 2 days. The animals were treated i.p. with ACM on day 10 after tumor inoculation and their PC were harvested 4 days later for the CL assay.

Treatments. Aclacinomycin, a cytotoxic antibiotic isolated from cultures of Streptomyces galilaeus, was kindly supplied by Laboratoires Roger Bellon (Neuilly-sur-Seine, France). For use, the drug was dissolved in a 0.9% NaCl solution at a concentration of 1 mg/ml. This solution, stored at −20°C, was used within 8 days. In all the following experiments, 4 mg/kg were injected i.p. 4 days before harvesting. This dose was selected because it stimulates the CL and the antitumoral PC activity and prolongs the survival of tumor-bearing mice (5, 11). Control mice received saline.

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1 To whom requests for reprints should be addressed, at ICIG-Hôpital Paul-Brousse, 14, av. P. V. Couturier, 94800-Villejuif [A. B-C., J. C., S. O-A.], and Unité 25, Institut National de la Santé et de la Recherche Médicale, Hôpital Necker, 75015-Paris [F. H-D., France.

2 The abbreviations used are: ACM, aclacinomycin; AA, arachidonic acid; A 23187, Ca²⁺ ionophore A 23187; CL, chemoluminescence; NDA, nordihydroguaiaretic acid; PC, peritoneal cells; PMA, 4α-phorbol-12-myristate-13-acetate; PG, prostaglandin; RIA, radioimmunoassay; SOD, superoxide dismutase; TXB₂, thromboxane B₂.
Reagents. Indomethacin, nordihydroguaiaretic acid, superoxide dismutase, catalase, zymosan, Ca^{++} ionophore A 23187, 4-phorbol-12-myristate-13-acetate, and potassium cyanide were purchased from Sigma Chemical Co. Prostaglandin E \(_2\), indomethacin, and NDGA were dissolved in ethanol and diluted in Hanks' solution without phenol red. The enzymes and KCN were dissolved in phenol red-free Hanks' solution at pH 7.2 before utilization.

A 23187 and PMA were dissolved in dimethyl sulfoxide, 1 mg/ml, and kept frozen at −20°C in the dark. Further dilutions were performed in Hanks' solution without phenol red.

Tritiated prostaglandins (>100 Ci/m mole) were purchased from Amersham Center (United Kingdom) and nonradioactive prostaglandins were purchased from Sigma Chemical Co. Anti-prostaglandins \(E_2\) and \(F_2\) \_ and thromboxane \(B_2\) antisera were obtained from Pasteur Institute (France) and anti-6-keto-PGF\(_{1\alpha}\) antisera was purchased from Biosys (France). The organic solvents used in the extraction of prostanoiids were obtained from Baker (Netherlands).

Measurement of Macrophage Chemoluminescence. PC were suspended at a concentration of 10°/ml in phenol red-free Hanks' solution. This suspension contained 27–34% macrophages and 3–5% neutrophils. CL measurements were performed in an LKB 1250 luminometer at 37°C in a light-tight chamber with 1 ml of PC suspension (12). Twenty ¼ l of Lumilog (Sigma Chemical Co.) were added at a final concentration of 5 \times 10\(^{-4}\) M. When background light emission became constant, different stimuli were added (100 ¼ l of a suspension of opsonized zymosan (15 mg/ml), 10 ng/ml of PMA, or 5 ¼ g/ml of A 23187), and photomission was recorded for 60 min. A curve was drawn recording the emitted CL, expressed in mV, as a function of incubation time, expressed in minutes, and its area was calculated to facilitate their representation. The results presented in this work are the mean values ± SD of values obtained and are expressed in mV\cdot min.

For these experiments we used 10 mice/group and individual tests were performed. Some experiments were performed using a pool of peritoneal cells from 10 normal or ACM-treated mice. Under these conditions, each test was performed in triplicate and similar results were obtained.

Extraction of Prostaglandin Fractions and Radioimmunoassay for PG. Briefly, 2 \times 10^5 peritoneal macrophages, identified by neutral red from normal or ACM-treated mice, were incubated in 0.6 ml of Hanks' solution without phenol red at 37°C for 60 min either with 60 ¼ l of zymosan or without the activator. An additional sample was incubated in the presence of indomethacin (10\(^{-4}\) M final concentration) and zymosan. After incubation, the suspensions were centrifuged at 400 \(\times\) g for 5 min and the supernatants were harvested and the zymosan-elicited CL response was measured.

Chemoluminescent Response in Relation to Volume of ACM Injection. Recent clinical studies (17) have indicated the feasibility of peritoneal tumor treatment by i.p. drug perfusion, but the volume of drug administration appears to play an important role in the efficiency of the treatment. The present experiment was designed to show whether the volume in which a given dose of ACM is injected is also a factor of some importance in the stimulation of PC. Thus, groups of mice received i.p. injections of the same dose of ACM (4 mg/kg) in different volumes of saline (0.1, 0.5, 1, and 2 ml). Four days later, the PC were harvested and the zymosan-elicited CL response was measured.

The response increases with the volume increases; when the drug is given in a volume of 0.1 ml, the response is 87 ± 7 mV\cdot min; in a volume of 0.5 ml, it is 180 ± 20 mV\cdot min; in a volume of 1 ml, it is 231 ± 18 mV\cdot min; and in a volume of 2 ml, it is 293 ± 20 mV\cdot min.
of 1 ml, it is 250 ± 25 mV·min, which is the highest response. The stimulation decreases to 80 ± 13 mV·min when the volume of ACM injection is 2 ml.

After the injection of 0.1 or 0.5 ml of saline, the response of the PC is comparable to that of cells from untreated mice (36 ± 5 mV·min). However, after the injection of 1 or 2 ml of saline, the values are 30% greater, suggesting possible evidence of some mechanical stimulation in the peritoneal cavity. Therefore, the volume in which ACM is injected plays an important role in the stimulation of CL emission of PC.

Effect of Antioxidant Agents and Potassium Cyanide on CL Response. In order to evaluate the participation of 

\[ \text{OH}^- \text{, } \text{O}_2^- \text{, and } \text{H}_2\text{O}_2 \]

in the CL generation, to zymosan-triggered normal or ACM-treated PC were added ethanol (20 μM), an OH· scavenger, superoxide dismutase (300 units/ml), which transforms \( \text{O}_2^- \) into \( \text{H}_2\text{O}_2 \), or catalase (2750 units/ml), which transforms \( \text{H}_2\text{O}_2 \) into \( \text{H}_2\text{O} \) (18).

The results reported in Fig. 3 indicate that, under our experimental conditions, among the oxygen-free radicals liberated by the phagocytic cells after zymosan triggering, \( \text{O}_2^- \) and \( \text{H}_2\text{O}_2 \) are responsible for the CL phenomenon, as it may be concluded from the high inhibition of the CL response in the presence of SOD or catalase. The participation of OH· is limited, as seen by the lack of inhibition following the addition of ethanol. The CL response of normal cells is sensitive to the same inhibitors as is that of ACM-treated cells.

To evaluate the participation of mitochondrial respiration in the CL response of ACM-treated PC, we measured it in the presence or absence of KCN (100 μM final concentration), which is a potent inhibitor of the mitochondrial respiratory chain (19). Fig. 3 also shows that, while ACM-treated PC emit more CL than control cells, no alteration in intensity or in kinetics can be obtained with KCN addition.

Participation of Arachidonic Acid Metabolism in CL of ACM-treated PC. Since AA metabolites have been implicated in CL of normal phagocytic cells (7, 8), we measured CL in the presence of different concentrations of indomethacin or NDGA. At low concentrations indomethacin is a specific inhibitor of the cyclooxygenase pathway and, at high concentrations, of both cyclooxygenase and lipoxygenase pathways. NDGA is a more selective inhibitor of the lipoxygenase pathway (20). This assay was performed on normal and ACM-treated unfractionated PC populations, on plastic-adherent PC (containing 80% of macrophages) and on PC depleted of macrophages by carbonyl iron phagocytosis.

The addition of \( 10^{-9} \) or \( 10^{-6} \) M indomethacin to unfractionated and to plastic-adherent PC at the time of the zymosan triggering had a slight stimulatory effect, both on normal and on ACM-treated PC populations. At \( 10^{-3} \) M, indomethacin has a strong inhibitory effect (Table 1A); in contrast, macrophage-depleted PC do not emit CL, as the values obtained are quite comparable to background values.

The addition of \( 7 \times 10^{-7} \) and \( 7 \times 10^{-9} \) M NDGA has a potent inhibitory effect on CL emission by both normal and ACM-treated PC populations (Table 1B). The viability of the cells was not modified by addition of either indomethacin or NDGA at all concentrations tested, as judged by trypan blue exclusion.

These results confirm the participation of AA metabolism products on the development of CL emission and suggest that the lipoxygenase pathway largely participates in this phenomenon in normal and treated cells.

Attempts at Modulating the CL of ACM-treated PC by Prostaglandins. Prostaglandins, mainly those of the E series, may have, under some conditions, a regulatory activity on CL (21). The following experiment was planned to study the effects of the addition of PGE2 and 6-keto-PGF1α on the CL emission of normal or ACM-treated PC.

PGE2 added at \( 10^{-6} \) M final concentration to unfractionated or plastic-adherent PC partially inhibits the CL response both in normal and ACM-treated PC populations triggered with zymosan. When added at \( 10^{-4} \) M final concentration, no effect was observed (Table 1C). 6-Keto-PGF1α added to the cells under the same experimental conditions had comparable effects (data not shown).

Prostaglandin Production by Peritoneal Macrophages after ACM Injection. Macrophages are well known to produce PG when they are triggered by the stimuli which trigger CL emission (22). We have therefore measured the production of PGE2, PGF2α, 6-keto-PGF1α, and TXB2 in the supernatant of normal or ACM-treated cells. The results in Table 2 show that the peritoneal macrophages of both groups produce important amounts of PG when stimulated with zymosan. The normal and ACM-treated cells secreted comparable amounts of PGE2 and 6-keto-PGF1α spontaneously or after zymosan treatment. However, ACM-treated macrophages secreted 2.5–3.8-fold more PGF2α and TXB2, respectively, than normal macrophages spontaneously and under triggering conditions. On the other hand, the PG production by normal or treated peritoneal cells is strongly inhibited by \( 10^{-6} \) indomethacin. These results confirm the activation of AA metabolism by zymosan. On the other hand, when macrophage-depleted PC were similarly treated, the PG production was minimal in all of the groups.

Chemoluminescent Response of PC from Tumor-bearing Mice. The studies reported above were done on PC from normal mice. To approximate the clinical situation, ACM stimulatory capacity was also evaluated on PC from tumor-bearing mice. Consequently, mice were given s.c. injections in the left foot of \( 1 \times 10^4 \) L1210 leukemic cells. Ten days later, ACM (4 mg/kg) was injected i.p. and the CL of the PC was measured 4 days later (Table 1D). ACM injection enhances 2.5-fold the CL of whole PC from tumor-bearing mice and 3.5-fold that of non-treated tumor-bearing mice as compared to normal nontreated mice; similar results were obtained with plastic-adherent cells.
ENHANCEMENT OF PERITONEAL CELL CHEMOLUMINESCENCE BY ACM

Table 1  Zymosan-elicited chemoluminescence of unfractonated and fractionated peritoneal cells from normal or ACM-treated mice (4 mg/kg i.p., day -4)

Effect of different in vivo and in vitro treatments. CL is emitted by 1 x 10^9 whole cells. Adherent cells were obtained by plastic adherence of the unfractonated PC containing 3 x 10^9 macrophages. Macrophage-depleted PC (3 x 10^9) were prepared by carbonyl iron phagocytosis. Each experiment was repeated at least three times. The values obtained with macrophage-depleted PC in each group are comparable to values given by zymosan alone without cells (1.5 ± 0.9).

<table>
<thead>
<tr>
<th>In vitro treatments for A-C</th>
<th>Control</th>
<th>ACM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unfractionated PC</td>
<td>Adherent PC</td>
</tr>
<tr>
<td>A. Indomethacin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>30 ± 4</td>
<td>29 ± 2</td>
</tr>
<tr>
<td>10^{-6}</td>
<td>41 ± 3</td>
<td>39 ± 4</td>
</tr>
<tr>
<td>10^{-3}</td>
<td>57 ± 5</td>
<td>42 ± 5</td>
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<tr>
<td>B. NDGA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>10 ± 2</td>
<td>6 ± 2</td>
</tr>
<tr>
<td>7 x 10^{-4}</td>
<td>12 ± 2</td>
<td>9 ± 1</td>
</tr>
<tr>
<td>7 x 10^{-4}</td>
<td>6 ± 0.7</td>
<td>4 ± 1</td>
</tr>
<tr>
<td>C. PGE_2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>33 ± 4</td>
<td>28 ± 2</td>
</tr>
<tr>
<td>7 x 10^{-4}</td>
<td>28 ± 4</td>
<td>26 ± 3</td>
</tr>
<tr>
<td>7 x 10^{-4}</td>
<td>17 ± 2</td>
<td>12 ± 2</td>
</tr>
<tr>
<td>D. Tumor-bearing mice (L1210)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>33 ± 4</td>
<td>29 ± 3</td>
</tr>
<tr>
<td>+</td>
<td>18 ± 3</td>
<td>12 ± 4</td>
</tr>
</tbody>
</table>

Table 2  Prostaglandin production by peritoneal macrophages from normal or ACM-treated mice (4 mg/kg day -4)

Prostaglandins were measured by RIA in the supernatant of peritoneal macrophages triggered or not triggered with zymosan (60 μl) during 60 min of incubation at 37°C in 5% CO₂. Results are expressed as pg/0.5 ml/2 x 10^9 cells and are means ± SD of 4 independent experiments. Each test was done in triplicate. Column 6 refers to the control experiment done with 2 x 10^9 macrophage-depleted PC incubated with zymosan. Student's r: between A and A', /' = 0.05; between B and B', P = 0.02; between C and C', P < 0.005. Only statistically significant differences are indicated.

<table>
<thead>
<tr>
<th>Experimental conditions of cell incubation</th>
<th>Zymosan + inomethacin 10^{-6} M</th>
<th>% of inhibition by indomethacin</th>
<th>Macrophage-depleted by zymosan</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Spontaneous</td>
<td>Zymosan</td>
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<tr>
<td>PGE_2</td>
<td>50 ± 20</td>
<td>1147 ± 262</td>
<td>51 ± 16</td>
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<tr>
<td>ACM</td>
<td>73 ± 19</td>
<td>1139 ± 255</td>
<td>35 ± 26</td>
</tr>
<tr>
<td>6-Keto-PGF_1α</td>
<td>465 ± 87</td>
<td>4241 ± 1639</td>
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<tr>
<td>ACM</td>
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<td>4091 ± 3449</td>
<td>195 ± 64</td>
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<tr>
<td>PGF_2α</td>
<td>18 ± 2 (B)</td>
<td>66 ± 8 (C)</td>
<td>11 ± 6</td>
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<tr>
<td>ACM</td>
<td>38 ± 16 (B')</td>
<td>164 ± 8 (C')</td>
<td>15 ± 8</td>
</tr>
<tr>
<td>TXB_2</td>
<td>199 ± 116 (A)</td>
<td>815 ± 36 (C)</td>
<td>142 ± 60</td>
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<tr>
<td>ACM</td>
<td>733 ± 249 (A')</td>
<td>3134 ± 87 (C')</td>
<td>335 ± 109</td>
</tr>
</tbody>
</table>

DISCUSSION

We have previously reported (5) that the respiratory burst triggered in phagocytic cells from ACM-treated mice is enhanced, as has been described (23) for other anthracelines, but the biochemical events or the physical factors which participate in the production of free radicals in those cells after anthracline administration are not well documented.

Under our experimental conditions, the macrophages are the cells mainly responsible for the CL emission of PC, as we have reported (5). We confirmed that plastic-adherent PC containing 80% of macrophages in all experiments respond similarly to unfractionated PC. On the contrary, when PC depleted of macrophages by carbonyl iron phagocytosis were assayed, no light emission was detected.

To emit CL, phagocytic cells may be variously triggered using zymosan, which stimulates the respiratory burst by activation of the Fc receptors (24): PMA, which directly stimulates the protein C kinase by substitution of the diacylglycerol (25); or Ca²⁺ ionophore A 23187, which increases the influx of intracellular Ca²⁺ without stimulating the protein C kinase (26).

In the present study, we show that these three agents generate a greater CL in PC from ACM-treated mice than in PC from normal mice, thus indicating that the enhanced CL of PC from ACM-treated mice can be seen through different respiratory burst-generating pathways but not through a particular one.

The modality of ACM administration is of great importance in relation to the intensity of PC response. We have previously observed that the antitumor response against L1210 or P815 cells and CL emitted by PC is increased when ACM is given i.p. but not when given i.v. or i.m. Similar observations have been made with other anthracelines concerning antitumoral activity (27, 28). In the present paper, we report that the volume in which the drug is given also influences the response. These findings could be attributed to the concentration of the drug. We know that the CL and antitumoral ACM response of treated mice are dose dependent (5). In addition, the volume may also

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interfere by allowing better drug distribution in the peritoneal cavity.

The second part of this work was devoted to the analysis of the biochemical events implicated in CL of untreated and ACM-treated PC. We show that the enhanced ACM-induced CL emission is associated with a high liberation of O$_2^-$ and H$_2$O$_2$ since CL may be inhibited by SOD and catalase, respectively. Those active oxygen derivatives could participate in tumor cell lysis and have microbicidal activity (1, 2). Since KCN does not modify the intensity of the CL emission of ACM-treated cells, mitochondrial respiration is not involved; it is suggested that we are dealing with a membrane phenomenon, as has been reported (19) in normal phagocytic cells.

On the other hand, our data indicate an important participation of AA metabolism in the CL of ACM-treated PC through the lipoxygenase pathway, since the response can be inhibited by NDGA (at 7 x 10$^{-9}$ and 7 x 10$^{-8}$ M) and by indomethacin at a high concentration (10$^{-4}$ M).

It is well demonstrated (20) that zymosan stimulates the production of PG. Preferential release of TXB$_2$ over PGE$_2$ in zymosan-triggered normal cells has also been reported (29, 30). Our observations on normal and ACM-treated macrophages are in complete agreement with these findings. Moreover, under our experimental conditions ACM-treated peritoneal macrophages produced significantly more PGF$_2$ and TXB$_2$ than normal cells, indicating a modification of AA metabolism in ACM-treated cells. The inhibition of PG secretion by indomethacin (10$^{-4}$ M), together with a slight stimulatory effect on CL emission, suggests that cyclooxygenase derivatives do not act as mediators in the CL induction in our model, but more probably as negative feedback inhibitors, as demonstrated by the addition of exogenous PG and in agreement with the results reported by others (7, 8, 21) in normal cells. However, the effect of indomethacin (10$^{-4}$ M) may also be the result of a better availability of AA for the lipoxygenase pathway.

Finally, the last point to stress is that ACM stimulates PC both from normal and from tumor-bearing mice. If the latter cells appear to emit slightly less CL, this may be due to tumor-induced immunodepression, which is well documented (31).

In conclusion, the ACM stimulates the CL of PC. This enhanced CL is dependent upon the liberation of oxygen-free radicals and on products of AA metabolism. It can be obtained under conditions close to clinical situations and is affected by the same parameters which affect the efficiency of i.p. chemotherapy (17). It is possible that the efficiency of i.p. chemotherapy and anthracycline-induced PC stimulation might be correlated. On the other hand, ACM appears to be a very promising candidate for the i.p. therapy of peritoneal tumors such as ovarian carcinomas (32).

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