REGULATION OF MACROPHAGE LYSOSOMAL ENZYME SECRETION: ROLE OF ARACHIDONATE METABOLITES, DIVALENT CATIONS AND CYCLIC AMP

RODGER M. McMILLAN,* D. EUAN MACINTYRE,† JULIAN E. BEESLEY‡ AND JOHN L. GORDON

University Department of Pathology, Tennis Court Road, Cambridge, U.K., and ARC Institute of Animal Physiology, Babraham, Cambridge, U.K.

SUMMARY

We have investigated the role in macrophage lysosomal enzyme release of arachidonate metabolites, extracellular divalent cations and cyclic AMP (cAMP) which modulate secretion in other cell types. Lysosomal enzyme secretion induced by zymosan was accompanied by release of malondialdehyde (MDA), which is derived from arachidonic acid via prostaglandin synthase. Blockade of MDA formation, by aspirin or indomethacin, was associated with only a small inhibitory effect on lysosomal enzyme release by zymosan: arachidonate metabolites thus play only a minor role in mediating macrophage lysosomal enzyme release.

Zymosan-induced secretion of lysosomal enzymes from macrophages did not require extracellular magnesium or calcium although release was enhanced by magnesium and inhibited by calcium. These effects may be related to an influence of the ions on phagocytosis. Elevation of intracellular divalent cation concentrations, by ionophore A23187, induced release of lysosomal enzymes but this was a result of cell lysis.

Adenosyl cyclase stimulants and dibutyryl cAMP produced slight inhibition of zymosan-induced lysosomal enzyme release. Aminophylline and papaverine caused more marked inhibition but their effects may be due to actions independent of phosphodiesterase inhibition.

Our data indicate that arachidonate metabolites and cAMP do not play a major role in regulating zymosan-induced enzyme release from macrophages. Extracellular calcium and magnesium may modulate secretion but the role of intracellular divalent cations remains to be established. We conclude that macrophage lysosomal enzyme secretion is controlled by regulatory mechanisms different from those which control similar degranulation processes in other cell types.

INTRODUCTION

Peritoneal macrophages in culture release lysosomal enzymes in response to phagocytic stimuli (Weissmann, Dukor & Zurier, 1971), but the mechanisms that regulate macrophage lysosomal enzyme secretion are not fully understood. Polymorphonuclear leukocytes also release lysosomal enzymes during phagocytosis (Weissmann et al. 1971) and the mechanisms that control this secretory process are well documented:

* Present Address: Dartmouth Medical School, Department of Medicine, Connective Tissue Disease Section, Hanover, N.H., 03755 U.S.A.
† Present Address: Department of Surgery, Harvard Medical School, Beth Israel Hospital, Boston, MA. 02115, U.S.A.
‡ Present Address: Wellcome Research Laboratories, Beckenham, Kent, U.K.
inhibitors of prostaglandin (PG) synthesis and agents that elevate cellular cAMP levels inhibit lysosomal enzyme release, whereas elevation of intracellular divalent cation concentrations by ionophore A23187 stimulates enzyme secretion (Weissmann et al. 1971; Smith, 1978; Smith & Ignarro, 1975). These agents have similar actions on secretion from blood platelets and mast cells and this has led to the proposal of a common mechanism of stimulus–secretion coupling in inflammatory cells (Henson, 1974).

Macrophage lysosomal enzyme release has many similarities to secretion from polymorphonuclear leukocytes and it is tempting to suggest that the 2 processes might be regulated in the same way. At present there are few experimental data either to support or to disprove this proposal. In this study we have therefore investigated the characteristics of lysosomal enzyme release from macrophages induced by a phagocytic stimulus, zymosan, and we have studied the importance, to macrophage secretion, of changes in divalent cation concentrations, prostaglandin synthesis and cAMP levels.

MATERIALS

All tissue culture materials were supplied by Flow Laboratories, Irvine, U.K. Arachidonic acid, aminophylline, cAMP, dibutyryl cAMP, indomethacin, adrenaline and NADH were obtained from Sigma Chemical Company, Poole, U.K.; thiobarbituric acid was from Aldrich Chemical Company, Gillingham, U.K. and 4-methyl-umbelliferyl-2-acetamido-2-deoxy-β-D-glucopyranoside was from Koch-Light Laboratories, Colnbrook, U.K. Aspirin (acetyl salicylic acid) and papaverine were obtained from Addenbrooke’s Hospital Pharmacy, Cambridge, U.K., Ionophore A23187 was a gift from Lilly Research Centre, U.K. Prostaglandin E1, 15(S)-hydroxy-9,11α-epoxymethano-prostaglandin acid (U44069) and 15α-hydroxy-11α,9α-epoxymethano prostaglandin acid (U46619) were generously provided by Dr J. E. Pike, Upjohn Company, Michigan, U.S.A.

METHODS

Preparation of macrophages

Male Balb/c mice were killed by cervical dislocation and the abdominal fur was soaked with 70% ethanol then peeled back to expose the peritoneum. A mixture of 3.5 ml Hanks’ Balanced Salt Solution (HBSS) and 1.5 ml air was injected into the peritoneum and, following gentle massage, fluid was withdrawn using the same syringe and needle. A recovery of around 3 ml was usually achieved and this contained 2–3 x 10⁶ cells. Samples contaminated with red blood cells were discarded and the remaining cell suspensions were pooled and stored at 4 °C in plastic universal containers. Pooled peritoneal exudates were centrifuged (600 g; 10 min), and the cell pellet was resuspended in minimal essential medium (MEM) containing 20 mM HEPES buffer, heat-inactivated foetal bovine serum (20% v:v), 45 μg/ml sodium bicarbonate, 2 mM glutamine and 50 μg/ml gentamycin.

The cells were incubated for 3 h at 37 °C in polystyrene multiwell plates. The medium was then decanted, and the cells were rinsed vigorously with phosphate-buffered saline, pH 7.4 (PBS) and fresh culture medium was added. The following day dead and non-adherent cells were removed by rinsing with PBS. This method yielded an almost pure macrophage monolayer. Cells were routinely used at a density of 3–5 x 10⁶/well (16 mm diameter).

Stimulation of macrophage secretion

Suspensions of zymosan, the stimulus that was normally used for inducing macrophage lysosomal enzyme release, were prepared according to the method of Weissmann et al. (1971). Stock suspensions (10 mg/ml) were stored at 4 °C. On the day of use, a subsample of the
Macrophage lysosomal enzyme secretion

zymosan suspension was washed a further 3 times in PBS before dilution to the required concentration.

Stimulation of secretion from macrophages was routinely performed in serum-free medium which contained 0.1 % (w:v) Lactalbumin Hydrolysate. For studies on the role of extracellular divalent cations, cells were incubated in HBSS without calcium and magnesium, to allow addition of known concentrations of these ions. Because of contaminants present in other components this HBSS was not totally free of calcium and magnesium, although the concentration was < 1 % of that in conventional HBSS. Attempts to reduce this further with EDTA or EGTA were unsuccessful because the chelators caused cell damage during prolonged incubation. Macrophages were routinely used the day after preparation. Culture medium was decanted, the cell layer was rinsed with PBS, and fresh serum-free medium was added. Cells were normally incubated with drugs for 30 min at 37 °C before addition of zymosan. Incubations were continued for a further 4 h, after which samples of the culture supernatant were removed for biochemical analysis. Intracellular analyses were performed after digesting the cells with 0.2 % Triton X-100.

Measurement of lysosomal enzyme release

Lysosomal enzyme activity was measured by a fluorimetric technique based on the release of 4-methylumbelliferone, developed by modification of the technique of Gordon (1975) for measuring platelet lysosomal enzymes. β-N-acetyl glucosaminidase (β-NAG) was chosen as the marker enzyme. The reaction mixture contained 50 μl of culture supernatant or cell digest; 50 μl of 0.3 M citrate buffer pH 4.9; 50 μl of 3 mM, 4-methylumbelliferyl-2-acetamido-2-deoxy-β-D-glucopyranoside.

The reaction mixtures were incubated at 37 °C in a polystyrene microtitration plate. After 1 h, a subsample (50 μl) was added to 1.5 ml glycine-NaOH buffer (50 mM glycine in 39 mM NaOH; pH 10.4). Fluorescence, in 1-ml samples, was measured in a Farrand Mk 1 Spectrophotofluorimeter using excitation and emission wavelengths of 370 and 450 nm respectively (uncorrected instrument readings). Release of β-NAG was expressed as a percentage of the intracellular enzyme activity.

Measurement of malondialdehyde (MDA) release

MDA, which is derived from arachidonic acid via PG synthase, was measured fluorimetrically (McMillan, MacIntyre & Gordon, 1977). Samples of culture supernatant (0.4 ml) were mixed with equal volumes of 0.5 % thiobarbituric acid and either incubated overnight at room temperature or heated at 70 °C for 30 min. The mixtures were diluted with 0.4 ml distilled water and fluorescence was measured in 1-ml samples using excitation and emission wavelengths of 515 and 553 nm respectively (uncorrected instrument readings).

Measurement of lactate dehydrogenase (LDH) release

As an index of cell lysis, release of LDH (a cytoplasmic enzyme) was determined photometrically by a modification of the method of Wroblewski & LaDue (1955). The reaction mixture comprised 0.1 ml culture supernatant or cell digest plus 125 μg/ml NADH, 125 μg/ml sodium pyruvate and 0.1 M PBS in a final volume of 0.4 ml. LDH activity was measured as the rate of decrease of absorbance at 340 nm due to oxidation of NADH. Photometric measurements were made in a Pye Unicam SP 1800 spectrophotometer. Release of LDH was expressed as a percentage of the intracellular enzyme activity.

Electron microscopy

For transmission electron-microscope studies, cells were fixed in situ with glutaraldehyde (2.5 % solution in 0.05 M sodium cacodylate buffer containing 0.05 M CaCl₂ and 0.05 M NaCl, at pH 7.2) and postfixed with 1 % osmium tetroxide in the same buffer. Cells were block stained with either 2 % aqueous uranyl acetate or 2 % aqueous tannic acid then dehydrated by sequential treatment with 70, 90 and 100 % ethanol. The culture dishes were then treated with propylene oxide; this dissolved the polystyrene below the cells and allowed the monolayer to float free.
The cells were rinsed with propylene oxide, embedded in Araldite, and sectioned on an LKB Ultratome. Light gold–silver sections were stained with uranyl acetate (ethanolic, saturated) and Reynold's lead citrate. Samples were viewed in a JEOL 100C transmission electron microscope at 80 kV.

RESULTS

Characteristics of zymosan-induced secretion of lysosomal enzymes from macrophages

Incubation of mouse peritoneal macrophages with zymosan for 4 h at 37 °C produced concentration-dependent release of the acid hydrolase β-NAG from the macrophage lysosomes. This was a selective process: incubation of the cells with 500 μg/ml zymosan caused over 50% release of β-NAG with very little release of LDH (Fig. 1). For studies with potential inhibitors of lysosomal enzyme secretion, a submaximal concentration of zymosan (50 μg/ml) was routinely used.

Lysosomal enzyme secretion from macrophages stimulated by zymosan was a slow process: little release occurred within 30 min but longer incubations resulted in progressive accumulation of β-NAG in the culture supernatant: around 12% release after 2 h, and up to 80% release after 24 h (Fig. 2). The kinetics of lysosomal enzyme
release from macrophages reflected the slow rate of uptake of zymosan by the cells. Fig. 3 shows light micrographs of peritoneal macrophages incubated for different times with zymosan. Attachment of particles to cells was evident within 30 min but no ingestion of zymosan was apparent (Fig. 3A). Longer incubation periods resulted in ingestion of zymosan by the cells (Fig. 3B, C). As the cells became filled with zymosan particles they lost their well-spread, flattened appearance, and after 24 h all the macrophages were swollen and rounded (Fig. 3C). There was also a decrease in the number of adherent cells, suggesting a loss of cell viability. In subsequent experiments macrophages were therefore routinely incubated with zymosan for 4 h, which resulted in substantial release of lysosomal enzymes without apparently affecting cell viability.

**Role of arachidonate metabolites as mediators of macrophage lysosomal enzyme secretion**

Incubation of macrophages with zymosan produced parallel, concentration-dependent secretion of β-NAG and MDA, an indicator of cyclo-oxygenase activity (Fig. 4). Aspirin or indomethacin, which inhibit cyclo-oxygenase, reduced zymosan-induced MDA formation by over 90% but caused less than 25% inhibition of lysosomal enzyme secretion (Fig. 5).
Fig. 3. Effect of zymosan on morphology of peritoneal macrophages. Mouse peritoneal macrophages were incubated with zymosan (50 μg/ml) at 37 °C for (A) 20 min, (B) 4 h, and (C) 24 h, after which the cells were rinsed with PBS and fixed in 2.5 % glutaraldehyde.
Fig. 4. Release of MDA and \( \beta \)-NAG from macrophages induced by zymosan. Mouse peritoneal macrophages were incubated with a range of zymosan concentrations for 4 h at 37 °C after which subsamples of culture supernatant were removed for determination of MDA release (○) and \( \beta \)-NAG release (●). Points represent mean values ± S.E.M. (n = 8).

Fig. 5. Effect of cyclo-oxygenase inhibitors on MDA and \( \beta \)-NAG release from macrophages induced by zymosan. Mouse peritoneal macrophages were incubated for 30 min at 37 °C alone (C), with aspirin (10\(^{-3}\) M) (A), or indomethacin (10\(^{-4}\) M) (I) before addition of zymosan. Incubations were continued for a further 4 h after which subsamples of culture supernatant were removed for determination of MDA release (□) and \( \beta \)-NAG release (◼). Results are expressed as percentages of release in the absence of cyclo-oxygenase inhibitors. Histograms represent mean values ± S.E.M. (n = 8).
Addition of arachidonic acid, the major endogenous substrate for macrophage cyclo-oxygenase, induced release of $\beta$-NAG at concentrations of 10–100 $\mu$M; this was accompanied by considerable LDH release, indicating that cell lysis rather than selective secretion was responsible. Structural analogues of the prostaglandin endoperoxide PGH$_2$ (compounds U44069 and U46619) did not induce significant lysosomal enzyme release from macrophages at concentrations up to 10 $\mu$M (Table 1).

Table 1. Enzyme release from macrophages induced by arachidonic acid and stable endoperoxide analogues

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>Enzyme release, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NAG</td>
</tr>
<tr>
<td>Zymosan, 50 $\mu$g/ml</td>
<td>14.3 ± 1.1</td>
</tr>
<tr>
<td>Arachidonic acid, 10 $\mu$M</td>
<td>12.7 ± 1.06</td>
</tr>
<tr>
<td>Arachidonic acid, 33 $\mu$M</td>
<td>17.2 ± 1.48</td>
</tr>
<tr>
<td>Arachidonic acid, 100 $\mu$M</td>
<td>53.3 ± 4.46</td>
</tr>
<tr>
<td>U44069, 10 $\mu$M</td>
<td>0.20 ± 0.02</td>
</tr>
<tr>
<td>U46619, 10 $\mu$M</td>
<td>1.37 ± 0.08</td>
</tr>
</tbody>
</table>

Cells incubated with stimuli for 2 h at 37 °C. Values represent means ± S.E.M. (n = 4). NT = not tested.

Fig. 6. Effect of divalent cations on zymosan-induced lysosomal enzyme release from macrophages. Mouse peritoneal macrophages were incubated for 30 min at 37 °C in HBSS with or without added calcium (5 mM) or magnesium (5 mM) as chloride salts. Zymosan (50 $\mu$g/ml) was added and the incubations continued for a further 4 h. Release of $\beta$-NAG is expressed as percentage of intracellular enzyme levels. Histograms represent mean values ± S.E.M. (n = 5).
Fig. 7. Effect of divalent cations on morphology of peritoneal macrophages. Mouse peritoneal macrophages were incubated for 4 h at 37 °C in the following media: A, calcium- and magnesium-free Hanks balanced salt solution (HBSS); B, HBSS + calcium (5 mM); C, HBSS + magnesium (5 mM); D, HBSS + calcium (5 mM) + magnesium (5 mM). Cells were fixed in 2.5% glutaraldehyde. × 200.
Role of divalent cations as mediators of macrophage lysosomal enzyme release

Zymosan-induced secretion of lysosomal enzymes from macrophages occurred in the absence of extracellular calcium and magnesium but addition of divalent cations influenced secretion: magnesium enhanced release and calcium was inhibitory. Release in the presence of equimolar concentrations of extracellular calcium and magnesium was similar to that in the absence of both ions (Fig. 6).

![Graph showing enzyme release from macrophages induced by A23187.](image)

Fig. 8. Enzyme release from macrophages induced by A23187. Mouse peritoneal macrophages were incubated for 2 h at 37 °C with a range of concentrations of A23187. Subsamples of the culture supernatant were removed for measurement of β-NAG (●—●) and LDH (○—○). Results, which represent mean values ± S.E.M. (n = 3), are expressed as percentages of intracellular enzyme levels.

The morphology of peritoneal macrophages was sensitive to changes in the divalent cation composition of the extracellular medium. Cells incubated in the absence of divalent cations, or in the presence of calcium alone, were mainly rounded in appearance. Addition of magnesium, or of calcium and magnesium, induced the macrophages to spread out on the surface of the culture dishes (Fig. 7).

Ionophore A23187 produced concentration-dependent release of β-NAG from macrophages. Release of β-NAG by A23187, however, was always accompanied by leakage of LDH, indicating that cell lysis rather than selective lysosomal enzyme secretion had occurred (Fig. 8). This was confirmed by ultrastructural studies: Fig. 9 shows macrophages incubated with or without 3 μM ionophore A23187, then stained with tannic acid after fixation. In control cells this stains the plasma membrane (including surface invaginations) intensely but, since tannic acid does not readily cross intact cell membranes, cytoplasmic and nuclear constituents are less intensely...
Fig. 9. Effect of ionophore A23187 on ultrastructure of mouse peritoneal macrophages. Mouse peritoneal macrophages incubated for 2 h at 37 °C with (a) 0.1% dimethyl sulphoxide or (b) 3 µM A23187 in 0.1% dimethyl sulphoxide. Cells were fixed with glutaraldehyde and stained with tannic acid. Arrow indicates intense staining of plasma membrane in control cell. × 12200.
stained (Fig. 9A). Following exposure to A23187 there is no clear demarcation of the cell membrane and there is marked cytoplasmic disruption (Fig. 9B).

**Modulation of macrophage lysosomal enzyme release by cAMP**

Table 2 shows the effect on macrophage lysosomal enzyme release of exogenous cAMP and of agents that elevate cellular concentrations of cAMP either by activating adenylate cyclase (e.g. adrenalin, PGE$_1$) or by inhibiting phosphodiesterase (e.g. papaverine, aminophylline).

<table>
<thead>
<tr>
<th>Drug</th>
<th>Concentration, M</th>
<th>β-NAG release, % control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dibutyryl cAMP</td>
<td>5 x 10^{-4}</td>
<td>90 ± 5</td>
</tr>
<tr>
<td>Dibutyryl cAMP</td>
<td>10^{-3}</td>
<td>73 ± 6</td>
</tr>
<tr>
<td>Dibutyryl cAMP</td>
<td>5 x 10^{-3}</td>
<td>71 ± 3</td>
</tr>
<tr>
<td>cAMP</td>
<td>5 x 10^{-3}</td>
<td>101 ± 5</td>
</tr>
<tr>
<td>Adrenalin</td>
<td>10^{-7}</td>
<td>98 ± 6</td>
</tr>
<tr>
<td>Adrenalin</td>
<td>10^{-8}</td>
<td>86 ± 8</td>
</tr>
<tr>
<td>Adrenalin</td>
<td>10^{-9}</td>
<td>74 ± 4</td>
</tr>
<tr>
<td>Prostaglandin E$_1$</td>
<td>10^{-7}</td>
<td>92 ± 5</td>
</tr>
<tr>
<td>Prostaglandin E$_1$</td>
<td>10^{-4}</td>
<td>74 ± 2</td>
</tr>
<tr>
<td>Prostaglandin E$_1$</td>
<td>10^{-3}</td>
<td>71 ± 3</td>
</tr>
<tr>
<td>Papaverine</td>
<td>10^{-4}</td>
<td>83 ± 7</td>
</tr>
<tr>
<td>Papaverine</td>
<td>10^{-5}</td>
<td>52 ± 3</td>
</tr>
<tr>
<td>Papaverine</td>
<td>10^{-6}</td>
<td>29 ± 5</td>
</tr>
<tr>
<td>Aminophylline</td>
<td>10^{-4}</td>
<td>86 ± 5</td>
</tr>
<tr>
<td>Aminophylline</td>
<td>10^{-3}</td>
<td>64 ± 3</td>
</tr>
<tr>
<td>Aminophylline</td>
<td>10^{-8}</td>
<td>8 ± 2</td>
</tr>
</tbody>
</table>

Cells incubated with drugs for 30 min at 37 °C before addition of zymosan (50 µg/ml). Values represent means ± S.E.M. (n = 5).

Dibutyryl cAMP caused concentration-dependent inhibition of zymosan-induced lysosomal enzyme release, with maximum inhibition (~ 30%) at concentrations of 1 mM and above. cAMP at a similar concentration was ineffective. PGE$_1$ and adrenalin also produced concentration-dependent inhibition of macrophage lysosomal enzyme release: both hormones inhibited by about 30% at a dose of 10 µM. No further inhibition was produced by higher concentrations of the hormones; indeed addition of higher doses of adrenalin to macrophage cultures resulted in cell lysis. Aminophylline and papaverine were more effective inhibitors of lysosomal enzyme release. High concentrations of aminophylline (> 1 mM) could abolish zymosan-induced secretion but at this concentration aminophylline interfered with attachment and spreading of the cells. Papaverine, which produced no morphological changes at concentrations up to 100 µM, inhibited zymosan-induced lysosomal enzyme release by approximately 70%.
DISCUSSION

Lysosomal enzymes are released from macrophages during phagocytosis of non-digestible particles (e.g. zymosan) but the mechanisms that govern secretion have not been determined. Degranulation processes in other cells such as platelets and granulocytes are apparently similar and have been shown to involve interactions between arachidonate metabolites, divalent cations and cAMP (Henson, 1974; MacIntyre, 1976). In the present study we have investigated the importance of these putative intracellular messengers in macrophage lysosomal enzyme release. Incubation of peritoneal macrophages with zymosan, a suspension of yeast cell wall particles, produced selective release of β-NAG, a lysosomal enzyme, without leakage of cytoplasmic constituents. Secretion was concentration- and time-dependent and the kinetics were consistent with the slow rate of particle uptake as indicated by light microscopy.

Phagocytosis is not essential for macrophage lysosomal enzyme release since soluble stimuli such as phorbol myristic acetate and activated complement components also induce secretion (Bonney et al. 1978a; Schorlemmer & Allison, 1976). Our own results indicate that secretion induced by phorbol ester is (like the response to zymosan) slow, progressive and accompanied by stimulation of arachidonate metabolism but by little or no LDH release (McMillan, unpublished). In addition, during prolonged culture peritoneal macrophages spontaneously release considerable amounts of lysosomal enzymes (Schnyder & Baggiolini, 1978). In contrast to degranulation this process requires protein synthesis and presumably represents a different mechanism of secretion.

Lysosomal enzyme release by zymosan was accompanied by secretion of products derived from arachidonic acid via cyclo-oxygenase: in this study we demonstrated MDA release from macrophages during phagocytosis, and other workers have reported that zymosan stimulates secretion of prostaglandins, thromboxanes and prostacyclin (Humes et al. 1977; Brune, Glatt, Kalin & Peskar, 1978; Stringfellow, Fitzpatrick, Sun & McGuire, 1978).

Studies with a range of zymosan concentrations demonstrated that MDA release paralleled secretion of β-NAG. Metabolism of arachidonic acid also accompanies lysosomal enzyme release in blood platelets and we have demonstrated that cyclooxygenase products (viz. PG endoperoxides and thromboxane A₂) are necessary for secretion of these enzymes from platelets (MacIntyre, McMillan & Gordon, 1977). Two observations from the present study suggest that, in contrast, arachidonate metabolites do not have a major role in mediating macrophage lysosomal enzyme release. Firstly, arachidonic acid and synthetic analogues of prostaglandin endoperoxides did not induce selective release of lysosomal enzymes from macrophages. Secondly, aspirin or indomethacin produced little inhibition of lysosomal enzyme secretion at concentrations that virtually abolished MDA formation. These data are consistent with the work of Bonney et al. (1978b), who reported that indomethacin abolished prostaglandin release without affecting secretion of lysosomal acid hydrolases.
Translocation of divalent cations, particularly calcium, plays a central role in regulating secretion from several inflammatory cells. This may occur either by mobilization of intracellular ions, as in blood platelets (Feinman & Detwiler, 1974) or by facilitating entry of extracellular cations, as in mast cells (Foreman, Mongar & Gomperts, 1973). In the present study we found that zymosan-induced lysosomal enzyme secretion does not have an absolute requirement for extracellular divalent cations. However, secretion was enhanced by extracellular magnesium and inhibited by calcium. Similarly, spreading of the cells in culture, which represents phagocytosis of an infinite surface (North, 1968), was dependent on magnesium but not calcium; this is in agreement with the findings of Rabinovitch (1975). It is probable that the influence of extracellular divalent cations on lysosomal enzyme release and on spreading is a reflection of their effects on phagocytosis: uptake of opsonized erythrocytes by macrophages is also known to require magnesium but not calcium (Henson, 1969; Lay & Nussenzweig, 1968).

Although extracellular calcium and magnesium are not essential for macrophage lysosomal enzyme release, it is possible that the secretory process might be mediated by intracellular divalent cations. In order to investigate this possibility, ionophore A23187 was employed as a release inducer. This compound translocates divalent cations by mobilizing intracellular ion pools (Pressman, 1973) and induces selective secretion of granule constituents from platelets, mast cells and neutrophil granulocytes. We found that A23187 stimulated dose-dependent release of lysosomal enzymes from macrophages but biochemical and ultrastructural studies showed that release was a consequence of cell lysis. Thus, in contrast to similar secretory processes in other cells, mobilization of divalent cations alone is not a sufficient stimulus for macrophage lysosomal enzyme secretion.

Weissmann et al. (1971) suggested that elevation of cellular cAMP concentrations inhibits release of lysosomal enzymes from phagocytes. In our experiments, high concentrations of dibutyryl cAMP inhibited zymosan-induced lysosomal enzyme release from macrophages by only 30%. cAMP was not active, presumably because it is a much more polar compound and therefore enters cells less readily. Prostaglandin E1 or adrenalin inhibited zymosan-induced lysosomal enzyme secretion by 20–30%; the concentrations that caused maximum inhibition (in each case around 10 μM) are in good agreement with those that maximally stimulate adenylate cyclase activity in macrophage homogenates (Remold O'Donnell, 1974).

Welscher & Cruchaud (1976) reported that agents which elevate cAMP inhibit substantially lysosomal enzyme release from macrophages induced by immune complexes. The reason for this divergence is not clear although it may be related to the different secretory stimulus used. However, our results are consistent with those of Ringrose, Parr & McLaren (1975), who found PGE1 had only a small inhibitory effect on zymosan-induced lysosomal enzyme secretion at concentrations up to 100 μM.

The small inhibitory effects of prostaglandin E1, adrenalin and dibutyryl cAMP suggest either that cAMP has only a minor role in regulating macrophage lysosomal enzyme release, or that the cells have a high basal level of phosphodiesterase activity which opposes the potential elevation of cAMP concentrations produced by cyclase.
Macrophage lysosomal enzyme secretion

stimulation. In order to clarify this point we studied the effect on secretion of 2 phosphodiesterase inhibitors. High doses of aminophylline blocked zymosan-induced secretion completely and papaverine produced up to 70% inhibition. These results suggest that a phosphodiesterase might be important in controlling macrophage cAMP levels, but this concept is not supported by other experimental data: prostaglandins and catecholamines increase cAMP concentration in macrophages but theophylline, which resembles aminophylline both structurally and pharmacologically, is inactive (Gemsa, Steggemann, Menzel & Till, 1975; Welscher & Cruchaud, 1976). The activity of these drugs as inhibitors of lysosomal enzyme release might therefore be due to actions independent of cAMP metabolism. In the present study aminophylline caused morphological changes that indicate a possible cytotoxic effect; and papaverine, at the concentrations used here, has been shown to inhibit oxidative metabolism and to reduce ATP levels in isolated cells (Patriarca et al. 1973; Garland & Johansen, 1977). Further studies using phosphodiesterase inhibitors are necessary to determine the relationship between their effects on cAMP levels and on enzyme secretion. However, the balance of evidence, at present, suggests that cAMP has only a minor modulatory effect on macrophage lysosomal enzyme release and that the level of intracellular cAMP may be less important in regulating secretion in macrophages than in other cells such as platelets and granulocytes.

In summary, the data presented in this study demonstrate that arachidonate metabolites and cAMP do not play a major role in regulating zymosan-induced lysosomal enzyme secretion from macrophages. Secretion occurs in the absence of divalent cations but extracellular calcium and magnesium may modulate the process. The role of intracellular divalent cations remains to be clearly established. Thus, macrophage lysosomal enzyme release is not controlled by the same regulatory mechanisms as the degranulation processes in polymorphonuclear leukocytes, platelets, and mast cells. Lysosomal enzyme release from macrophages is a much slower process than secretion from the other cell types and this may be functionally very important. Prolonged enzyme release from macrophages is consistent with the major role of this cell in chronic inflammation. In contrast, mast cells, granulocytes and platelets, which contribute primarily to acute inflammation, exhibit rapid degranulation processes. It seems likely that arachidonate metabolites, divalent cations and cAMP control degranulation in acute inflammation but that novel secretory mechanisms exist in macrophages. Identification of these regulatory mechanisms and of agents that block them could provide a new approach for modulating the secretory activity of macrophages in chronic inflammation.

We are grateful to Dr Edward D. Harris, Jr., Dr Jeremy D. Pearson and Ms Carol A. Vater for discussions and for their constructive criticisms. Ms Robin Scribner typed the manuscript.

REFERENCES


Macrophage lysosomal enzyme secretion


(Received 24 September 1979 – Revised 17 December 1979)