The selective release of phospholipase A₂ by resident mouse peritoneal macrophages

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Resident mouse peritoneal macrophages have three phospholipase activities: a phospholipase A₂ active at pH 4.5, a Ca²⁺-dependent phospholipase A₂ active at pH 8.5 and a phosphatidylinositol-specific phospholipase C activity. When macrophages are exposed to zymosan in culture, the cellular activity of pH-4.5 phospholipase A₂ is diminished in a manner dependent on zymosan concentration and time of exposure, whereas the cellular activities of pH-8.5 phospholipase A₂ and phospholipase C remain unchanged. The depletion of pH-4.5 phospholipase A₂ activity from the cell is paralleled by a quantitative recovery of this activity in the culture medium in a manner similar to the cellular depletion and extracellular recovery of two lysosomal enzymes. This release is specifically elicited by an inflammatory substance such as zymosan, since macrophages incubated with 6 μm latex spheres retain pH-4.5 phospholipase A₂ activity and lysosomal enzyme activities intracellularly.

Cells of the mononuclear phagocyte system synthesize and release a number of inflammatory mediators into their percellular environment on appropriate stimulation with inflammatory substances (Davies & Bonney, 1979). These include oxygenated products of arachidonic acid derived from both the cyclo-oxygenase and lipoxygenase pathways. Large amounts of prostaglandins E₂ and I₂ are synthesized by peritoneal macrophages responding to inflammatory stimuli such as antigen–antibody complexes (Bonney et al., 1979), lymphokines (Gordon et al., 1976), phorbol myristate acetate (Bonney et al., 1980) and zymosan (Humes et al., 1977). Products of the lipoxygenase pathway synthesized by macrophages include 12-hydroxyeicosa-5,8,10,14-tetraenoic acid (Rigaud et al., 1979) and slow-reacting substance of anaphylaxis (Bach & Brashler, 1978; Orange et al., 1980), including one characterized as leukotriene C (Bach et al., 1980; Rouzer et al., 1980). The formation of many of these lipid-derived mediators is initially dependent on the activity of phospholipases. Until recently only one such enzyme, a phospholipase A₂, optimally active at acid pH, has been described in mononuclear phagocytes (Franson & Waite, 1973). Studies in this laboratory have established the presence in resident mouse peritoneal macrophages of two phospholipase A₂ activities with pH optima of 4.5 and 8.5 respectively (Wightman et al., 1981a) and a phospholipase C (Wightman et al., 1981c). At this time there is no clear indication which of these enzymes, alone or in concert, participate in the macrophage's response to inflammatory stimuli. In the present study we have measured the activity of these enzymes in macrophages after exposure to zymosan in culture. We report that, whereas the cellular activities of the pH-8.5 phospholipase A₂ and phospholipase C remain unchanged, the cellular activities of the pH-4.5 phospholipase A₂ are depleted from the cell in a manner linearly dependent on the amount of zymosan added and the time of exposure to this stimulus and are quantitatively recovered in the culture medium.

Materials and methods

Macrophages were collected from male Swiss mice by peritoneal lavage with M199 containing 1% heat-inactivated porcine serum, 20 units of heparin/ml, 100 units of penicillin/ml and 100 μg of streptomycin/ml. The cells were plated at 5 × 10⁶–6 × 10⁶ per 50 mm culture dish and allowed to adhere for 2 h at 37°C in an atmosphere of CO₂/air (1:19). The non-adherent cells were removed by washing the cell sheet in 5 vol. of phosphate-buffered saline. The adherent cells, which we have previously demonstrated by morphological and functional criteria to
be >95% macrophages, were maintained in serum-free medium M199 supplemented with 100 μg of gentamycin/ml.

Cultures maintained in 2 ml of serum-free medium M199 were incubated in the presence of the indicated amounts of zymosan particles or 6 μm latex spheres for 4 h (Humes et al., 1977). The culture medium was then removed and the cell sheet scraped into 2 ml of 0.01 M-Tris-buffered saline, pH 7.0. The suspension was briefly sonicated on ice and assayed for phospholipase activities with phospholipid substrates extracted from cultured mouse LM fibroblasts that had incorporated radioactive precursors of phospholipid (Wightman et al., 1981a). The phospholipase A₂ activities were assayed at their respective pH optima by methods previously described (Wightman et al., 1981a,b). Briefly, the pH-4.5 phospholipase A₂ activities were determined in a final volume of 80 μl of 0.1 M-acetate buffer, pH 4.5, containing 1 mM-EDTA, and were incubated at 37°C for 1 h. The pH-8.5 phospholipase A₂ activities were measured in a final volume of 80 μl of 0.1 M-Tris/HCl buffer, pH 8.5, containing 2 mM-Ca²⁺, and were incubated at 37°C for 18 h. The phospholipase A₂ reactions contained 6 nmol of [¹⁴C]arachidonic acid-labelled phospholipid added as a sonicated suspension and 10 μl of culture medium or sonicated cell suspension. The reactants and products were extracted (Bligh & Dyer, 1959) and purified on silica gel GF t.l.c. plates developed in chloroform/methanol/acetic acid/water (25:15:2:1, by vol.). [¹⁴C]Arachidonic acid hydrolysed was quantified by liquid-scintillation counting. Phospholipase C was assayed by measuring the release of phosphol[³H]inositol from [³H]phosphatidylinositol. Enzyme activity was determined in a final volume of 80 μl of 0.1 M-Tris/HCl buffer, pH 7.0, containing 1 mM-Ca²⁺. The reaction contained 8 nmol of [³H]phosphatidylinositol and 1–2 μg of macrophage homogenate protein. The reactions were terminated by extraction (Bligh & Dyer, 1959) and a portion of the aqueous phase was counted for released ³H. The phospholipase activity per culture is expressed as the number of nmol of [¹⁴C]-arachidonic acid released (for phospholipases A₂) or [³H]phosphorylinositol released (for phospholipase C) per h.

N-Acetyl-β-D-glucosaminidase was assayed by the method of Woolen et al. (1961) and β-glucuronidase by the method of Talalay et al. (1946). Lactate dehydrogenase was assayed by determining the rate of oxidation of NADH at 340 nm.

Results

Mouse peritoneal macrophages incubated in the presence of zymosan or latex spheres were assayed for their phospholipase activities. Fig. 1 shows that although the cellular pH-8.5 phospholipase A₂ and phospholipase C activities remain unchanged...

![Graph](image-url)

**Fig. 1.** Concentration-dependent depletion of pH-4.5 phospholipase A₂ from mouse peritoneal macrophages treated with zymosan

(a) shows cellular pH-4.5 phospholipase A₂; (b) shows cellular pH-8.5 phospholipase A₂; (c) shows cellular phospholipase C. * indicates those cellular enzyme activities that are significantly less than the untreated controls (P < 0.005).
whether exposed to zymosan or latex, the cellular pH-4.5 phospholipase A₂ activity is depleted in a concentration-dependent manner from cells treated with zymosan. Those cultures incubated with latex retain their pH-4.5 phospholipase A₂ activity intracellularly with no indication of extracellular release. Latex spheres are efficiently phagocytosed by macrophages but, unlike zymosan particles, they are not inflammatory (Humes et al., 1977).

The culture media from the untreated cells and from those cells that had been exposed to zymosan were assayed for pH-4.5 phospholipase A₂ activity. Fig. 2(a) shows that, as the pH-4.5 phospholipase A₂ is depleted from cells responding to zymosan, it is quantitatively recovered in the culture medium. The extent of this depletion and recovery are dependent on the amount of zymosan with which the macrophages were treated. Fig. 2(b) shows that this depletion from the cells and the accumulation in the culture medium are also dependent on the duration of exposure to zymosan.

The zymosan-induced release of pH-4.5 phospholipase A₂ from macrophages was then compared with the release of two lysosomal enzymes, N-acetyl-β-D-glucosaminidase and β-gluconidase in the same cultures. Lactate dehydrogenase, a cytoplasmic enzyme, was also assayed in these cultures. Fig. 3 shows that the zymosan-induced release of pH-4.5 phospholipase A₂ is paralleled by the release of the two lysosomal enzymes. Lactate dehydrogenase, however, is retained in the cell and is not released in response to this inflammatory stimulus. Both the intracellular and medium-associated lactate dehydrogenase activities remained constant regardless of exposure to zymosan or concentration of zymosan used, indicating a conservation of cell density throughout the course of the experiments. Therefore the translocation of lysosomal enzyme activities to the medium is one of specific release from the cell rather than selective detachment of cells as a result of ingesting zymosan. The zymosan-induced release of these enzymes is, therefore, selective and occurs in a manner dependent on the amount of zymosan added (Fig. 3a) and the duration of exposure (Fig. 3b) and suggests that the pH-4.5 phospholipase A₂ is of lysosomal origin.

**Discussion**

This significance of the release of pH-4.5 phospholipase A₂ by macrophages responding to inflammatory stimuli to the pathogenesis of inflammation remains to be resolved. Phospholipase A₂ activity is, however, known to accumulate in fluids associated with inflammatory exudates (Thouveton et al., 1974; Sahu & Lynn, 1977). Owens and his colleagues (Owens et al., 1980) have reported that a granulocyte phospholipase A₂ efficiently hydrolyses sarcolemma phospholipids,

![Fig. 2. Quantitative recovery in the culture medium of cellular pH-4.5 phospholipase A₂ depleted from macrophages after zymosan exposure](image)

![Fig. 3. The parallel release from macrophages of pH 4.5 phospholipase A₂ and of two lysosomal enzymes after exposure to zymosan](image)
lysophosphatides are important target stimuli. This phages of these pathogenesis proteinosis. Furthermore, with phosphatidylcholine products can play released by inflammation amounts of their pH producing, alveolar macrophages responding to inflammatory stimuli. Phospholipase A₂ activity has been described in glycogen-induced rabbit peritoneal exudate fluids (Franson et al., 1978) and its release has been characterized in polymorphonuclear leucocytes phagocytosing zymosan-complement (Authi & Traynor, 1979). Both of these activities are ascribed to the polymorphonuclear leucocyte and are similar in their pH optima (pH 6.0) and requirement for Ca²⁺ (10 mm).

We have characterized the phospholipase A₂ selectively released by resident mouse peritoneal macrophages exposed to zymosan. This activity is very different from those of the polymorphonuclear leucocyte (Authi & Traynor, 1979; Franson et al., 1974) in that it does not require Ca²⁺ for activity and is optimal at pH 4.5. The findings that we present complement those of other investigators (Muder et al., 1970; Authi & Traynor, 1979; Sirois et al., 1980), who have described phospholipase A₂ activities secreted under inflammatory conditions and that may be involved in the pathogenesis of these processes. The amount of pH 4.5 phospholipase A₂ secreted by the macrophage in response to zymosan is sufficient to produce 5 nmol of non-esterified arachidonic acid/h per culture. Macrophages under the same conditions produce 0.25 nmol of prostaglandins/h per culture (Bonney et al., 1978). However, whether the released pH 4.5 phospholipase A₂ can function in providing arachidonic acid to its oxygenation pathways or only complements the battery of macrophage enzymes that can function in tissue destruction remains to be elucidated.

References
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