

In vitro effects of hormones and autacoids on the activity of acid phosphatase in the lysates of endotoxin-activated rat peritoneal and bronchoalveolar macrophages

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Summary. Peritoneal and bronchoalveolar macrophages activated *in vitro* by endotoxin, exhibit alterations in the acid phosphatase activity of cell lysates when certain hormones or autacoids are present in the culture medium. They also show morphological changes concerning general appearance and acid phosphatase cytochemistry. Certain agents known to increase the intracellular levels of cyclic AMP, such as dopamine and prostaglandin E₂, decreased this enzyme activity in the lysates of peritoneal macrophages. Adrenalin had no effect on this activity at 14 hours, but was found to increase the activity in the culture medium at the initial hours of incubation. Glucagon decreased whereas insulin increased acid phosphatase activity in bronchoalveolar macrophages. Serotonin or histamine, known to activate phospholipase C, increased this activity in peritoneal or bronchoalveolar macrophages. The results of this study, taken together with previously published data (Kondomerkos et al., 2003), suggest that hormones and autacoids may control certain parameters of macrophage activation including acid phosphatase activity.

Key words: Macrophages, Acid phosphatase

Introduction

Macrophage activation is a process characterized by the enhancement of certain functional capabilities such as phagocytosis (Karnovsky and Lazdins, 1978; Adams and Hamilton, 1992). These cells possess a well-developed phagosomal-lysosomal apparatus that readily responds to the changes in the macrophage's environment (Cohn and Weiner, 1963; Cohn and Benson, 1965; Woodward, 1979; Cannon and Swanson,

1992). Macrophages express lysosomal acid phosphatase in order to degrade phagocytosed material (Radzun et al, 1982; Suter et al., 2001). The increased production of acid phosphatase or other lysosomal enzymes by activated macrophages has been well documented (Saito and Suter, 1965; Schnyder and Baggiolini, 1978; Reiner et al, 1981; Cooper et al., 1984; Henson et al., 1992). Macrophages exocytose acid phosphatase during phagocytosis but also independently of phagocytosis.

The morphology of macrophages depends on their functional status (Cooper et al., 1984; Adams and Hamilton, 1992). Their changes are evident by light or electron microscopy (Brederoo and Daems, 1972; Williams and Mayhew, 1973; Daems and Brederoo, 1973; Mayhew and Williams, 1974a,b; Morland and Kaplan, 1977). Acid phosphatase and other enzyme activities have been cytochemically demonstrated in various cell types of the monocyte-macrophage lineage (Yam et al., 1971; Snipes et al., 1986). The cytochemical location of acid phosphatase activity may vary markedly (Bainton and Golde, 1978).

We have previously shown that hormones and autacoids acting through cyclic AMP or phospholipase C, such as adrenalin, prostaglandin E₂, dopamine or serotonin, may control the hydrogen peroxide production and the morphology of lipopolysaccharide-activated macrophages (Kondomerkos et al., 2003). In this study we present biochemical and cytochemical evidence that these agents may also control the activity of acid phosphatase in activated macrophages.

Materials and methods

Chemicals

A23187 calcium ionophore (C-7522), Glucagon (G-9154), Histamine (H-7125), Insulin (I-6634), p-Nitrophenyl Phosphate Disodium Salt-Hexahydrate (N-4645), Phorbol 12-myristate-13-acetate (PMA, P-8139)

and Serotonin Hydrochloride (H-9523) were obtained from Sigma (Sigma-Aldrich Chemie GmbH, Munich Germany). The lipopolysaccharide (LPS) from *E. coli*, strain O55:B5, was also obtained from Sigma, either Phenol-extracted LPS (L-2880) or TCA-extracted LPS (L-4005). Adrenaline Hydrochloride (1 mg/ml solution, Lot. 98008) was purchased from DEMO Pharmaceuticals (DEMO Pharmaceuticals S.A., 14565 Krioneri, Greece). β -Glycerophosphate Disodium Salt-Pentahydrate (4168) and Malachite Green Oxalate (1.01398) were obtained from Merck (Merck KGaA, Darmstadt, Germany). Dexamethasone 21-Phosphate (Decadron) (4 mg/ml solution, Lot.4532) was from Vianex S.A. (Vianex, plant A', Athens, Greece). For cell culture, sterile 24-well culture plates (25820) manufactured by Corning were used (Corning BV Life Sciences, 1119 NE, Schipol-Rijk, The Netherlands). RPMI-1640 medium (F-1235), L-Glutamine (K-0282) and Fetal Calf Serum (FCS, S-0113) were provided by Seromed (Seromed/Biochrom AG, 12247 Berlin, Germany). Acid phosphatase cytochemistry was performed by using the Leukocyte Acid Phosphatase kit from Sigma (387-A). Stock solutions of A23187 and PMA were made in dimethyl sulfoxide (DMSO).

Animals and isolation of macrophages

The experiments were conducted in primary cultures of macrophages obtained from pathogen-free adult Wistar rats of both sexes, weighing approximately 180-210g each. These animals were handled as previously described (Kondomerkos et al., 2003). For each experiment three rats were used, all of the same litter. Once sacrificed, animals were immediately swabbed twice with 70% ethanol and isolation of either peritoneal or bronchoalveolar (bronchioloalveolar) macrophages was aseptically performed with modifications of standard methods as previously described (Cohn and Weiner, 1963; Cohn and Benson, 1965; McCarron et al., 1984; Kondomerkos et al., 2003).

Cell culture

After plating the cell suspension, each well was filled with 0.5 ml of RPMI-1640 medium containing 10% FCS and supplemented with 20 mM HEPES, 100.000 u/L penicillin, 100 mg/L streptomycin, 5.6 mg/L amphotericin-B and 0.3 g/L L-glutamine. Peritoneal or bronchoalveolar macrophages were allowed to adhere to the plates during a 2-hour incubation at 37 °C in a CO₂-incubator (5% carbon dioxide - 95% air environment). Then the wells were washed with warm PBS and replenished with 1 ml medium. Approximately 50% of the cells adhered to the plate (approximately 5x10⁵ cells/well). Phase-contrast observation with an inverted microscope revealed less than 2% contamination of the cell population with non-macrophage cells.

Acid phosphatase activity assay in the cell lysates

The determination of acid phosphatase activity in the macrophage lysates (intracellular enzyme activity) was based on previous methods for tissue homogenates (Appelmans et al., 1955; Gianetto and DeDuve, 1955; Hubscher and West, 1965). Macrophages were incubated for 14 hours with medium containing 2 μ g/ml lipopolysaccharide (LPS) and the effector agent, hormone or autacoid. The medium was aspirated from the cell wells and these wells were washed twice with warm 0.9% NaCl. To each well, 0.25 ml of cold lysis buffer (0.2% Triton X-100 in 0.05M acetate buffer pH 5.4) was added and the cell plates were placed on ice for 30 minutes. The lysates were diluted with 0.75 ml of warm 0.05 M acetate buffer and transferred to test tubes in a 37 °C water bath. To each tube, 1 ml of substrate solution containing 20 mM sodium β -glycerophosphate and 8 mM EDTA in 50 mM acetate buffer pH 5.4, was added. After a 60-minute incubation at 37 °C, the reaction was terminated by adding 0.095 ml of 70% perchloric acid and by chilling in an ice-bath for 15 minutes. All samples were centrifuged for 8 minutes at 700 g. Malachite green color reagent, 0.5 ml, was added to the supernatants (Baykov et al., 1988). The released inorganic phosphorus was measured colorimetrically at 630 nm. Results were expressed as micrograms (μ g) of phosphorus liberated per hour per 5x10⁵ cells \pm standard deviation. Enzyme activity was determined in pairs of one control and one experimental at a time. The "n" value (number of observations) signified the number of control-experimental pairs included in the results. Differences were evaluated by the Student's t-test and p values smaller than 0.05 were considered as significant (Hill, 1966).

Acid phosphatase activity assay in the culture medium supernatants

The determination of acid phosphatase activity in the supernatant culture medium (extracellular enzyme activity) was performed according to Schoene and Kamara (1999) and Aoyama et al (2001). From each well, 1 ml of medium was incubated with 0.02 ml of glacial acetic acid and 0.5 ml of 6 mg/ml solution of para-nitrophenyl-phosphate in 0.1 M acetate buffer for 65 minutes at 37 °C and pH 5.4. At 5 and 65 minutes, aliquots of 0.5 ml were transferred into tubes containing 5 ml of 1N NaOH. The liberated chromogen was determined colorimetrically at 405 nm.

Experimental design

The macrophages used in each experiment were obtained from rats of the same litter. Pooled cells from 3 rats were plated in 24-well culture plates. Each two adjacent rows of wells were used as "controls" and "experimentals". Each experiment included 6 to 12 control-experimental pairs. The experiment was usually

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repeated on different days. In experiments involving LPS, macrophages in control wells were incubated with 2 $\mu\text{g/ml}$ LPS, whereas macrophages in experimental wells were incubated with LPS plus the effector agent for 14 hours unless otherwise specified. The concentrations of adrenalin, serotonin, histamine and glucagon were 10^{-4} M, 5×10^{-5} M, 2×10^{-6} M and 10^{-7} M, respectively. Insulin concentration was 25 micrograms/ml (0.625 units/ml). The concentration of dexamethasone was 5×10^{-6} M. Calcium ionophore A23187 and PMA were used at concentrations of 1 μM . The results of the biochemical determinations were pooled for statistical analysis.

Light microscopy

For the study of general cell appearance and cytochemistry, macrophages were cultured directly on acid-treated and sterile glass microscopy slides placed inside Petri dishes. After a 24-hour incubation with culture medium containing 2 $\mu\text{g/ml}$ LPS and the agent under investigation (10^{-4} M adrenalin, 0.625 u/ml insulin or 5×10^{-6} M dexamethasone), the slides were washed three times in warm 0.9% NaCl. The cells were immediately fixed in a 3% solution of formaldehyde in a 68% solution of acetone in citrate buffer for 1 minute at room temperature. Then, they were washed with warm 0.9% NaCl and transferred to a staining jar (in a hot water bath, 37 °C) containing diazotized Fast Garnet GBC and Naphthol AS-BI phosphate in 0.1M acetate buffer, pH 5.4. After a 60-minute incubation, the slides were washed with warm 0.9% NaCl and counterstained with hematoxylin for 90 seconds. After rinsing briefly in running tap water, slides were air-dried and mounted with Entellan and a clear glass coverslip. Specimens were studied with a Leitz Ortholux-II microscope by two

independent observers. Photographs were taken at a magnification of x 1000 with a Leica DMLS microscope connected to a Sony Hi-Resolution CCD-IRIS Color Video Camera.

Results

Biochemical results

Initial experiments with peritoneal macrophages involved the determination of acid phosphatase activity in both the cell lysate and the supernatant culture medium, i.e. the intracellular and extracellular (exocytosed) enzyme activities. Cells were incubated with 2 $\mu\text{g/ml}$ LPS for 14 hours. Acid phosphatase activity in the lysate at 0, 3, 6 and 14 hours was: 1.194 ± 0.054 $\mu\text{g/hr}/5 \times 10^5$ cells, 1.064 ± 0.048 $\mu\text{g/hr}/5 \times 10^5$ cells, 1.209 ± 0.037 $\mu\text{g/hr}/5 \times 10^5$ cells and 1.453 ± 0.057 $\mu\text{g/hr}/5 \times 10^5$ cells, respectively (Fig. 1). Differences between sequential time points (i.e. 0-3, 3-6 and 6-14 hours) were statistically significant ($p < 0.01$). Activity in the supernatant at 0, 3, 6 and 14 hours was: 0.039 ± 0.024 $\mu\text{g/hr}/5 \times 10^5$ cells, 0.110 ± 0.029 $\mu\text{g/hr}/5 \times 10^5$ cells, 0.135 ± 0.025 $\mu\text{g/hr}/5 \times 10^5$ cells and 0.101 ± 0.022 $\mu\text{g/hr}/5 \times 10^5$ cells, respectively (Fig. 2). Differences in activity between sequential time points were statistically significant ($p < 0.05$).

To assess the participation of protein kinase C and ionized calcium in the development and exocytosis of acid phosphatase activity, peritoneal macrophages were incubated for 90 minutes in the culture medium containing either 1 μM phorbol myristate acetate (PMA) or 1 μM ionophore A23187. Phorbol myristate acetate, a protein kinase C activator, produced a non-significant increase ($p > 0.05$) in the cell lysate (intracellular activity). Calcium ionophore A23187 produced a

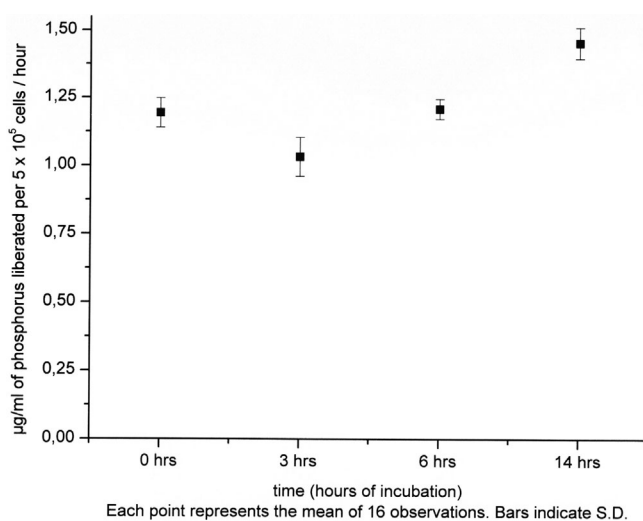


Fig. 1. Acid phosphatase activity in the lysate of LPS-activated peritoneal macrophages as a function of time.

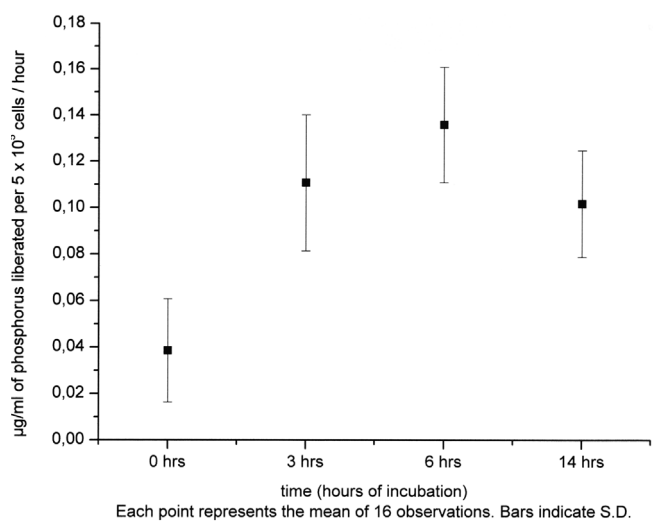


Fig. 2. Acid phosphatase activity in the supernatant of LPS-activated peritoneal macrophages as a function of time.

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significant increase (10.2%) in this activity (controls: $1.333 \pm 0.193 \mu\text{g/ml/hr}/5 \times 10^5$ cells; experimentals: $1.471 \pm 0.157 \mu\text{g/ml/hr}/5 \times 10^5$ cells, $n = 10$, $p < 0.05$) (Fig. 3). Both agents caused a significant increase in the supernatant culture medium (extracellular-exocytosed) enzyme activity (controls: $0.520 \pm 0.027 \mu\text{g/ml/hr}/5 \times 10^5$ cells; PMA-treated: $0.596 \pm 0.032 \mu\text{g/ml/hr}/5 \times 10^5$ cells, $n=10$, $p < 0.05$, controls: $0.520 \pm 0.027 \mu\text{g/ml/hr}/5 \times 10^5$ cells; A23187-treated: $0.685 \pm 0.049 \mu\text{g/ml/hr}/5 \times 10^5$ cells, $n=10$, $p < 0.01$) (Fig. 4).

In a further series of experiments, peritoneal macrophages incubated with $2 \mu\text{g/ml}$ of LPS for 14 hours, showed a 26.06 % decrease in acid phosphatase activity in lysates, as compared to cells incubated for the same time period without LPS (controls: 0.867 ± 0.130

$\mu\text{g/ml/hr}/5 \times 10^5$ cells; experimentals: $0.641 \pm 0.128 \mu\text{g/ml/hr}/5 \times 10^5$ cells, $n=34$, $p < 0.001$). Bronchoalveolar macrophages incubated with LPS also displayed a 26.75% decrease in acid phosphatase activity of the lysates, compared to cells incubated without LPS (controls: $0.826 \pm 0.142 \mu\text{g/ml/hr}/5 \times 10^5$ cells; experimentals: $0.605 \pm 0.117 \mu\text{g/ml/hr}/5 \times 10^5$ cells, $n=32$, $p < 0.001$).

Peritoneal macrophages incubated for 14 hours with $2 \mu\text{g/ml}$ LPS plus 2.5×10^{-5} M dopamine showed a decrease (52.02%) in acid phosphatase activity of the lysates, as compared to LPS-treated controls (controls : $0.594 \pm 0.058 \mu\text{g/ml/hr}/5 \times 10^5$ cells; dopamine-treated: $0.285 \pm 0.024 \mu\text{g/ml/hr}/5 \times 10^5$ cells, $n=10$, $p < 0.001$). Bronchoalveolar macrophages were not found to have differences in this activity (controls: $1.258 \pm 0.127 \mu\text{g/ml/hr}/5 \times 10^5$ cells; experimentals: $1.163 \pm 0.118 \mu\text{g/ml/hr}/5 \times 10^5$ cells, $n=12$, $p > 0.05$). Peritoneal macrophages incubated with LPS plus 10^{-5} M prostaglandin E2 (PGE2) showed a decrease (13.82 %) in this activity, as compared to controls (controls: $0.991 \pm 0.076 \mu\text{g/ml/hr}/5 \times 10^5$ cells; PGE2-treated: $0.854 \pm 0.088 \mu\text{g/ml/hr}/5 \times 10^5$ cells, $n=12$, $p < 0.001$). Peritoneal or bronchoalveolar macrophages incubated with LPS plus 10^{-4} M adrenalin showed no statistically significant decrease in this activity of the lysates (peritoneal controls: $0.928 \pm 0.120 \mu\text{g/ml/hr}/5 \times 10^5$ cells; adrenalin-treated: $0.876 \pm 0.127 \mu\text{g/ml/hr}/5 \times 10^5$ cells, $n=28$, $p > 0.05$; bronchoalveolar controls: $1.258 \pm 0.126 \mu\text{g/ml/hr}/5 \times 10^5$ cells; adrenalin-treated: $1.232 \pm 0.202 \mu\text{g/ml/hr}/5 \times 10^5$ cells, $n=12$, $p > 0.05$). (Table 1).

Incubation of peritoneal macrophages with LPS plus 10^{-7} M glucagon did not produce a statistically significant change in the acid phosphatase activity of lysates. However, bronchoalveolar macrophages showed a decrease (11.60%) in this activity as compared to

Table 1. The effects of dopamine, prostaglandin E2 and adrenalin on the activity of acid phosphatase in the lysates of LPS-activated peritoneal macrophages

EFFECTOR AGENTS	CONTROLS	EXPERIMENTALS	P
Dopamine	0.594 ± 0.058 (10)	0.285 ± 0.024 (10)	< 0.001
Prostaglandin E2	0.991 ± 0.076 (12)	0.854 ± 0.088 (12)	< 0.001
Adrenalin	0.928 ± 0.120 (28)	0.876 ± 0.127 (28)	> 0.05

Peritoneal macrophages were incubated for 14 hours with $2 \mu\text{g/ml}$ LPS and the effector agent (dopamine 2.5×10^{-5} M, prostaglandin E2 10^{-5} M or adrenalin 10^{-4} M). Acid phosphatase activity was determined in the cell lysate (intracellular activity). Results are means \pm S.D. and expressed as $\mu\text{g/ml}$ of phosphorus liberated per hour per 5×10^5 cells. Numbers in parentheses signify the number of observations (n) included in the results.

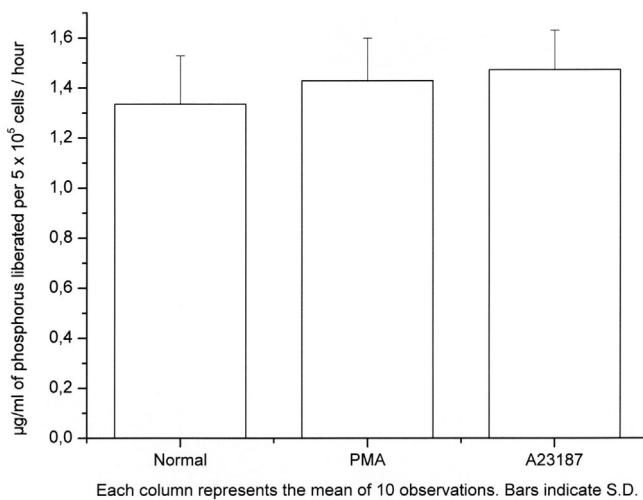


Fig. 3. Acid phosphatase activity in the lysate of peritoneal macrophages, 90 minutes after the addition of the effector agent.

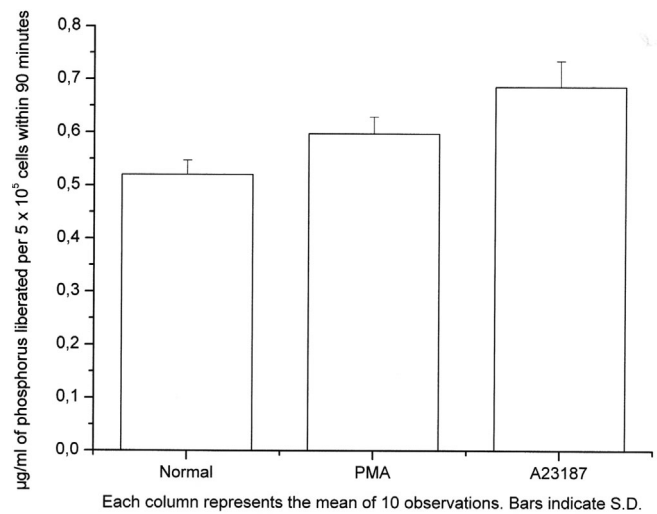


Fig. 4. Acid phosphatase activity in the supernatant of peritoneal macrophages, 90 minutes after the addition of the effector agents.

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controls. Peritoneal macrophages treated with LPS plus 25 $\mu\text{g/ml}$ (0.625 u/ml) insulin were not found to produce a significant effect on acid phosphatase activity of lysates. However, lysates of bronchoalveolar

macrophages showed an increase in acid phosphatase activity (11.63%) (Table 2).

Incubation of peritoneal macrophages with 2 $\mu\text{g/ml}$ LPS plus 5×10^{-5} M serotonin caused an 11.39% increase

Table 2. The effects of glucagon or insulin on acid phosphatase activity in the lysates of LPS-activated peritoneal or bronchoalveolar macrophages.

EFFECTOR AGENT	MACROPHAGE TYPE (number of observations)	CONTROL	EXPERIMENTAL	P
Glucagon	Peritoneal (22)	0.799 \pm 0.219	0.893 \pm 0.251	>0.05
	Bronchoalveolar (12)	0.448 \pm 0.022	0.396 \pm 0.035	<0.001
Insulin	Peritoneal (20)	0.864 \pm 0.054	0.856 \pm 0.076	>0.05
	Bronchoalveolar (12)	0.447 \pm 0.022	0.499 \pm 0.043	<0.001

Results are means \pm S.D. and expressed as $\mu\text{g/ml}$ of phosphorus liberated per hour per 5×10^5 cells. Numbers in parentheses signify the number of observations (n) included in the results.

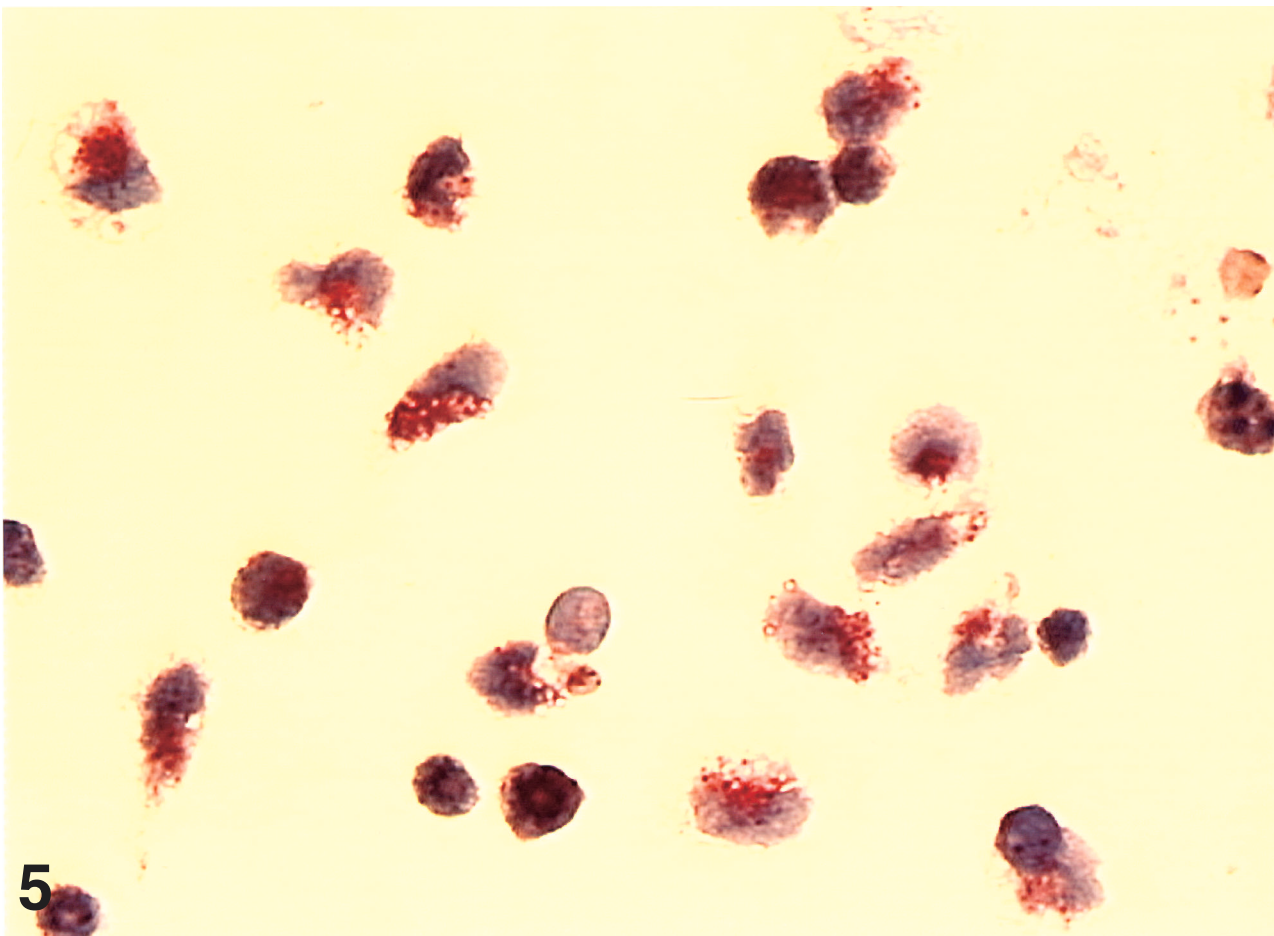


Fig. 5. Peritoneal macrophages incubated with LPS. Cytochemical reaction product for acid phosphatase is seen in regions of the cytoplasm. Hematoxylin, x 1000

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in acid phosphatase activity of lysates. Incubation of bronchoalveolar macrophages with this agent caused no statistically significant increase in this activity ($p>0.05$). Incubation of peritoneal macrophages with LPS plus

2×10^{-6} M histamine was not found to affect acid phosphatase activity in the cell lysates. However, histamine caused a marked increase (88%) in acid phosphatase activity in lysates of LPS-activated

Table 3. The effects of serotonin or histamine on acid phosphatase activity in the lysates of LPS-activated peritoneal or bronchoalveolar macrophages.

EFFECTOR AGENT	MACROPHAGE TYPE (number of observations)	CONTROL	EXPERIMENTAL	P
Serotonin	Peritoneal (22)	0.623±0.109	0.694±0.071	<0.05
	Bronchoalveolar (12)	0.225±0.042	0.251±0.087	> 0.05
Histamine	Peritoneal (27)	0.834±0.136	0.841±0.163	>0.05
	Bronchoalveolar (12)	0.225±0.042	0.423±0.093	< 0.001

Results are means±S.D. and expressed as $\mu\text{g/ml}$ of phosphorus liberated per hour per 5×10^5 cells. Numbers in parentheses signify the number of observations (n) included in the results.

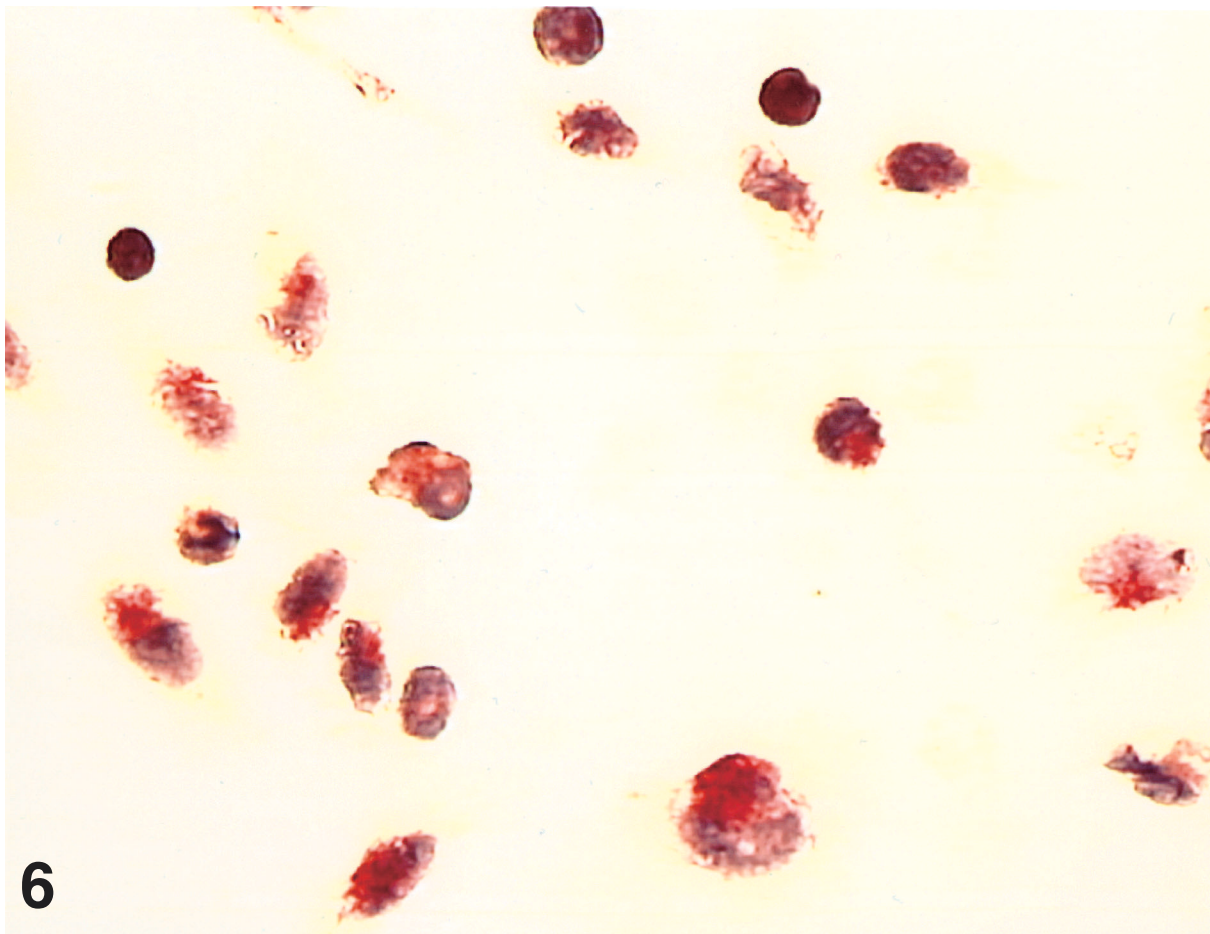


Fig. 6. Peritoneal macrophages incubated with LPS plus insulin. Cytochemical reaction product for acid phosphatase is rather strong and diffuse throughout their cytoplasm. Hematoxylin, x 1000

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bronchoalveolar macrophages (Table 3).

Peritoneal macrophages incubated with LPS plus 5×10^{-6} M dexamethasone showed an 19.96 % increase in acid phosphatase activity of the lysates, (controls: $0.576 \pm 0.077 \mu\text{g/ml/hr}/5 \times 10^5$ cells; dexamethasone-treated: $0.691 \pm 0.090 \mu\text{g/ml/hr}/5 \times 10^5$ cells, $n=24$, $p<0.001$).

Morphological results

Morphological changes produced by adrenalin, insulin or dexamethasone to the LPS-activated peritoneal macrophages cultured for 24 hours on acid-treated glass microscopy slides, are described.

Peritoneal macrophages incubated in culture medium were round or oval and varied in size. Their nucleus was kidney- or horseshoe-shaped. Many macrophages stained cytochemically for acid phosphatase activity, displaying a bright red pigment within their cytoplasm. The cytochemically-positive areas were well-defined, granular and usually located

within the groove (crease) of the kidney- or horseshoe-shaped nucleus. Macrophages with a round nucleus surrounded by a narrow margin of cytoplasm, showed no obvious area of acid phosphatase activity. Elongated cells showed faint or no activity.

Macrophages activated for 24 hours with $2 \mu\text{g/ml}$ LPS, displayed variable morphology. Most of the macrophages appeared rather large with abundant vacuolar cytoplasm and a large nucleus. They stained for acid phosphatase with a bright red pigment, sometimes granular and located in various regions of their cytoplasm (Fig. 5); compared to macrophages that had been cultured without LPS, the proportion of cells displaying a strong cytochemical reaction was smaller. Macrophages with a thin margin of cytoplasm surrounding a round nucleus did not usually reveal a cytochemical reaction.

Macrophages incubated with LPS plus 10^{-4} M adrenalin mostly appeared as small cells with scarce cytoplasm and a dark round or lobulated nucleus. There was only a thin rim of cytoplasm around the nucleus and

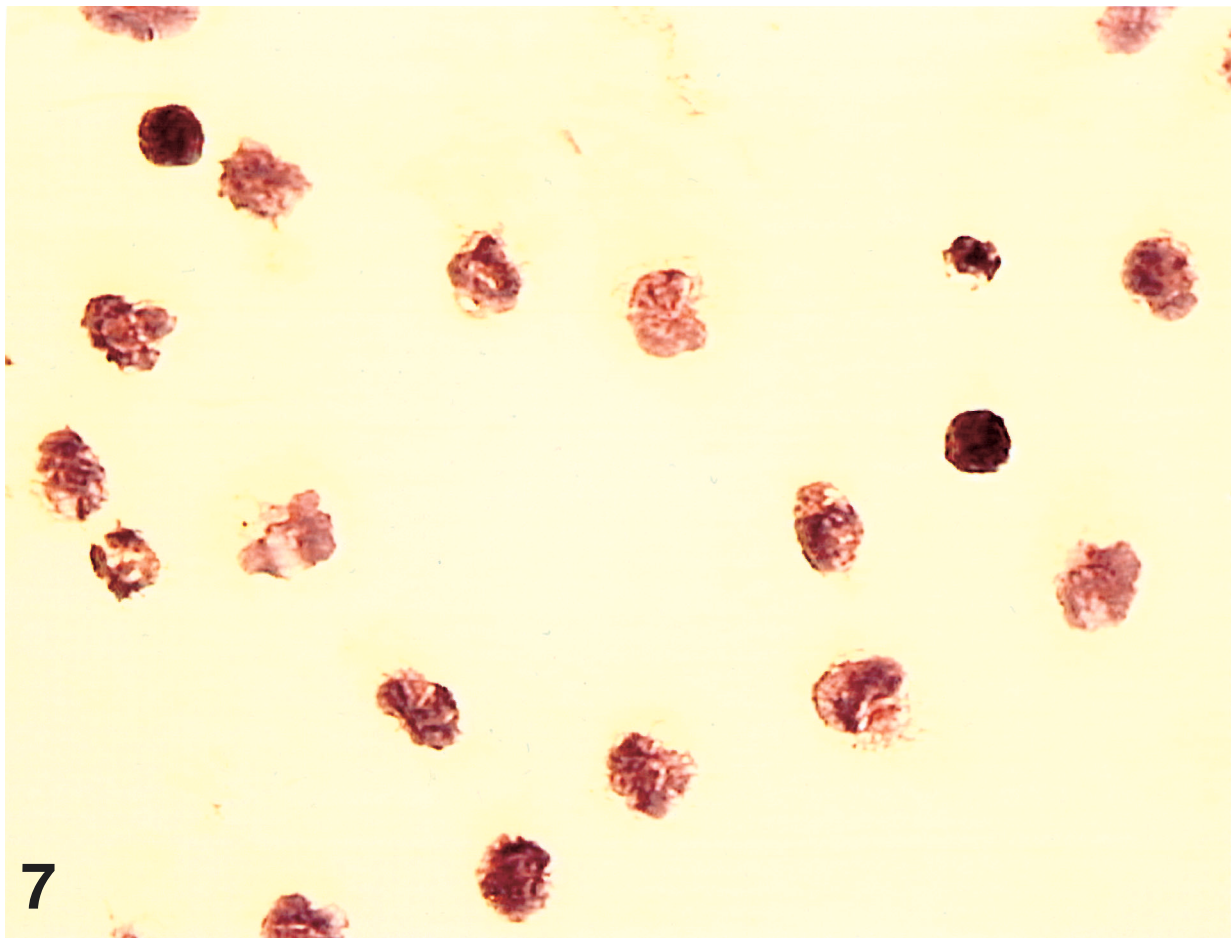


Fig. 7. Peritoneal macrophages incubated with LPS plus dexamethasone. Cytochemical reaction product for acid phosphatase is moderate in the cytoplasm. Hematoxylin, x 1000

the cytochemical reaction was difficult to estimate.

Macrophages incubated with LPS plus 0.625 units/ml of insulin mostly appeared as large cells, with abundant vacuolar cytoplasm and a large nucleus. The majority of these cells stained positively for acid phosphatase (Fig. 6). The reaction was rather strong. The cytochemically positive area was usually large and diffuse.

Macrophages incubated for 24 hours with LPS plus 5×10^{-5} M dexamethasone were large, round or polygonal. Their nucleus was also large and their cytoplasm appeared as a narrow margin around the nucleus. Some macrophages showed a strong, well-defined positive reaction for acid phosphatase (Fig. 7).

Discussion

The results of our initial experiments indicated that acid phosphatase activity in the lysates (intracellular activity) of LPS-activated macrophages initially decreased (at 3 hours) and then constantly increased from 3 to 14 hours of incubation; at 14 hours, acid phosphatase activity was even higher than the activity at 0 hours. Enzyme activity in the supernatant culture medium (extracellular-exocytosed activity) increased from 0 to 6 hours of incubation. These observations suggest that after activation with LPS, macrophages will initially exocytose their lysosomal content and then replenish their intracellular stores by new enzyme(s) with acid phosphatase activity, while continuing to exocytose a fraction of it. The stimulatory effects of phorbol myristate acetate and ionophore A23187 on intracellular or extracellular acid phosphatase activities suggest that protein kinase C and calcium participate in the processes of development and exocytosis of acid phosphatase activity.

Agents known to increase the cyclic AMP levels in macrophages, such as dopamine, prostaglandin E2 or adrenalin (Koopman et al., 1973; O'Donnell, 1974; Ikegami, 1977; Hunt et al., 1984; Spengler et al., 1989) decreased acid phosphatase activities in the lysates. Activation of the adenylate cyclase-PKA-CREB pathway moderates pro-inflammatory functions of macrophages, or leads to the production of anti-inflammatory substances that diminish the immune response (Guirao et al., 1997; Delgado et al., 1999). Dopamine is known to elevate cyclic AMP levels and its effects in macrophages may be mediated by dopaminergic or adrenergic receptors (Hasko et al., 1996, 2002). Prostaglandin E2 also affects cyclic AMP levels and PKA in macrophages, regulating various functions (O'Donnell, 1974; Metzger et al., 1981; Yamamoto and Suzuki, 1987). In a previous study, adrenalin, PGE2 and dopamine were found to control other important parameters of macrophage activation (Kondomerkos et al., 2003).

The inhibitory effect of glucagon on acid phosphatase activity in the lysates of macrophages is difficult to explain. Since glucagon produces no

elevation of cyclic AMP levels in macrophages (Smith et al., 1980), this effect may be due to the modulatory action of the hormone on the phospholipase C pathway, as has been described in other cells (Petersen and Bear, 1986; Wakelam et al., 1986; Murphy et al., 1987). Insulin, on the other hand, increased acid phosphatase activity in the lysates of bronchoalveolar macrophages. This hormone is known to lower cyclic AMP levels. The stimulatory effect of insulin on acid phosphatase activity may be related to its antagonistic action to glucagon (Pilkis et al., 1988). The presence of functional insulin receptors on macrophages has been proven and insulin has been reported to affect certain macrophage functions (Muschel et al., 1977; Abrass and Hori, 1984; Bermudez et al., 1990; Iida et al., 2001).

A modulatory effect of serotonin on activated macrophages, mediated by 5-HT₂ receptors, has been previously reported. These receptors activate phospholipase C, causing an increase in the cellular diacylglycerol and ionized calcium (Sternberg et al., 1986, 1987; Sanders-Bush and Mayer, 1996a,b). Generally, serotonin is considered to enhance immune function (Mössner and Lesch, 1998). A modulatory effect of histamine on bronchoalveolar macrophages, mediated by H₁-histaminergic receptors, has been previously reported (Cluzel et al., 1990; Vignola et al., 1994). These receptors either activate phospholipase C and increase cellular calcium and diacylglycerol, or elevate cyclic GMP and calcium (Ohno et al., 1991).

The stimulatory effect of dexamethasone on acid phosphatase activity in the lysates of peritoneal macrophages is difficult to explain. It may be due to increased enzyme synthesis, inhibition of enzyme release or recapture of exocytosed lysosomal content via mannose receptors (Ackerman and Beebe, 1975; Shepherd et al., 1985).

Some of the observed biochemical effects were only slight, although statistically significant. The biological significance of these changes remains to be investigated; agents that affect the same signal transduction pathway may act additively, contributing to more impressive changes in macrophage function.

The morphological and cytochemical alterations of macrophages observed in our study roughly corresponded to the biochemical changes. Morphological features of macrophage activation, such as abundant cytoplasm and a large nucleus (Kondomerkos et al., 2003), were abolished by adrenalin. The presence of cytochemical reaction for acid phosphatase was difficult to assess. Insulin showed opposite results. These alterations may reflect changes in the phagosomal-lysosomal apparatus of the cells. Insulin is known to activate phosphatidylinositol-3-kinase that mediates heterophagocytosis and the formation of endosomes in macrophages (Araki et al., 1996) and inhibits autophagocytosis in other cells (Kotoulas, 1981; Kalamidas and Kotoulas, 2000). Certain effects of insulin may also be due to its antagonistic action on the cAMP-PKA pathway (Muschel et al., 1977; Walkenbach

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et al., 1978; Heyworth and Houslay, 1983). The effects of dexamethasone cannot be explained by the results of this study; they may be possibly related to the ability of glucocorticoids to stabilize the lysosomal membrane.

Peritoneal and bronchoalveolar macrophages sometimes elicited different responses to the same effector agent. They apparently develop different phenotypes, due to their localization and other environmental factors. Peritoneal macrophages are situated in a sterile environment and will be immediately activated by exogenous stimuli; their bronchoalveolar counterparts are continuously exposed to airborne irritants and pathogens and develop self-inhibitory mechanisms to prevent damaging the alveoli. Functional heterogeneity of different types of macrophages has been previously described (Laskin et al., 1988; Turyna and Szuba, 1988; Gjomarkaj et al., 1999; Dorger et al., 2001; Fathi et al., 2001; Laskin et al., 2001).

Macrophages play an important role in the primary defense against intracellular and extracellular pathogens and neoplastic cells, participate in the immune response by antigen presentation and are involved in many physiological mechanisms and in the pathogenesis of certain diseases and their complications. Elucidation of the mechanisms that regulate macrophage function is important, as it improves our understanding of macrophage-related physiological processes, of the pathophysiology of macrophage-related diseases and their complications and finally may allow better therapeutic intervention. Agents secreted from the adrenal cortex and medulla during trauma, sepsis or other such conditions may be key mediators to suppressed or deficient macrophage function. The hormones and autacoids used in this study are present at sites of inflammatory or immune responses. The results of this study, taken together with previous data (Kondomerkos et al., 2003) indicate that such hormones and autacoids may control important parameters of macrophage activation, including acid phosphatase activity.

Acknowledgements. We thank Mr. Christos Economou and Miss Katerina Vourdas for expert technical assistance.

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Accepted May 30, 2003