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STIMULATION OF PHAGOCYTOSIS IN RAT POLYMORPHONUCLEAR LEUKOCYTES BY A23187 IS ACCOMPANIED BY ACTIVATION OF MYELOPEROXIDASE

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SUMMARY: The incubation of polymorphonuclear leukocytes from rats with A23187 resulted in stimulation of phagocytosis with a concomitant increase in the activity of myeloperoxidase. Activation of the enzyme by A23187 reached the maximum at 2.5 min of incubation and preceded the maximum stimulation of phagocytosis. Propylthiouracil, an inhibitor of myeloperoxidase, and Ca^{2+} chelators prevented activation of myeloperoxidase and phagocytosis by A23187. These results indicate that A23187 increases intracellular Ca^{2+} level, followed by activation of myeloperoxidase involved in stimulation of phagocytosis.

Phagocytosis is the most important defense mechanism in the animals. It is well established that myeloperoxidase (donor: hydrogen peroxide oxidereductase, EC 1.11.1.7) in the mature neutrophils is involved in the bactericidal function (1-5) among the defense system. In addition, myeloperoxidase prevented the opsonization (6,7). However, little is known about a role of myeloperoxidase in phagocytosis.

Myeloperoxidase, a marker of neutrophilic granulocytes, oxidizes a variety of compounds in the presence of hydrogen

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Abbreviations used: EGTA, ethylene glycol bis(β-aminoethylether)N,N,N',N'-tetraacetic acid; Quin-2, 2-[(2-amino-5-methylphenoxy)methyl]-6-methoxy-8-aminoquinoline-N,N,N',N'-tetraacetic acid; BAPTA, 1,2-bis(2-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid; PBS, phosphate-buffered saline; BCA, bicinchoninic acid.

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peroxide (8,9). This peroxidase is also concerned in oxidative metabolism of some compounds (10,11). Previously we have reported that the purification of myeloperoxidase from rat bone marrow and its enzymatic properties were well characterized and the properties of this enzyme was very similar to those of the enzyme obtained from mature leukocytes (12). In addition, expression of myeloperoxidase in the bone marrow cells was decreased during differentiation to the granulocytes (submitted). Thus we have shown that myeloperoxidase may play an important role in differentiation of bone marrow cells.

This study was designed to determine the enzyme activity during phagocytosis stimulated by A23187 to clarify a possible role of myeloperoxidase in phagocytosis. Present paper describes that stimulation of phagocytosis in polymorphonuclear leukocytes of rats was accompanied by activation of myeloperoxidase.

MATERIALS AND METHODS

Materials. A23187 was purchased from Calbiochem Co. FITC-Microsphere (Fluoresbright, 2.0 μ m diameter) was from Polysscience Inc. Bovine serum albumin was from Sigma Chemical Co. RPMI 1640 medium and fetal calf serum were from Flow Laboratories.

Preparation of the cells. Polymorphonuclear leukocytes were prepared from peritoneal exudates of casein (2 g/kg body weight) treated male Wistar rats, weighing about 250-300 g. The cells were extensively washed with phosphate buffered saline (PBS) and were hypoosmolytically treated to remove erythrocytes. The resulting cells were used throughout this experiment. These cells contained more than 90% of polymorphonuclear leukocytes.

Assay of phagocytosis. Phagocytosis was determined by flow cytometry according to Stewart et al. (13). Briefly, standard incubation mixture contained polymorphonuclear leukocytes (1 x 10^6 cells), fluorescent microspheres (1 x 10^8), RPMI 1640 and 5% fetal calf serum in a total volume of 1.0 ml. Unless otherwise indicated, the mixture was incubated at 37° C for 5 min. After the incubation the mixture was centrifuged at 100 x g for 8 min to separate the cells. Resulting cells were washed with the medium containing 2% BSA. This washing procedure were repeated 2 times to remove unphagocytized microspheres. The final cell suspension (2 x 10^4 cells)was analyzed by using flow cytometer (Epics 750).

Assay of myeloperoxidase activity. After the incubation without fluorescent microspheres, the cells were collected by

centrifugation at 100 x g for 8 min and washed with PBS for 3 times. Resulting cells were homogenized with 200 μ l of PBS by Polytron apparatus. This homogenate was used as the enzyme source. The activity of myeloperoxidase of polymorphonuclear leukocytes was determined as described previously (12).

Myeloperoxidase was homogeneously purified from rat bone marrow as described elsewhere (12).

Protein was determined by using BCA protein assay reagent (14).

RESULTS AND DISCUSSION

Fig. 1 shows the histograms of phagocytosis analyzed by flow cytometry. In addition to analysis of the percentage of the cells containing the microspheres, this procedure could be expanded into the determination of the numbers of phagocytic cells containing 1, 2, 3 and more than 4 microspheres as described by Stewart et al. (13). Each value of 0-12, 13-40, 41-66, 67-90 and 91-255 in channel number shows the numbers of phagocytic cells containing 1, 2, 3 and more than 4 microspheres, respectively (Fig. 1). Although unstimulated polymorphonuclear leukocytes possessed a low activity of phagocytosis, four-fold stimulation of phagocytosis was observed in the cells treated with 2.5 μ M A23187, an activator of the neutrophils. In addition, A23187 accelerated ingestion of not only one but also more than two microspheres. Therefore, this result explains that A23187 stimulates the activity of phagocytosis in polymorphonuclear leukocytes analyzed by flow cytometry using fluorescent microspheres.

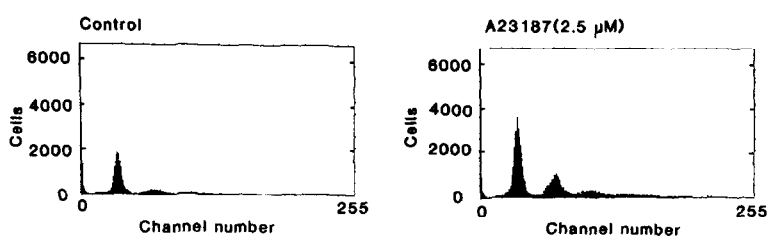

Fig. 1. Histograms of phagocytosis in control and A23187-stimulated polymorphonuclear leukocytes. The cells were incubated with fluorescent microspheres at 37° C for 5 min in the presence or absence of 2.5 μ M A23187. The cells were extensively washed with the medium containing 2% BSA after the incubation. The resulting cells (2 x 10^4 cells) were analyzed by flow cytometer.

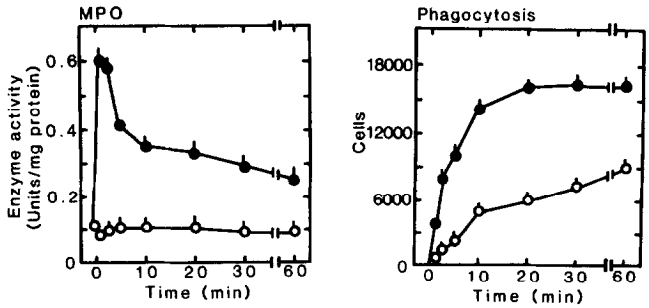

Fig. 2. Time courses of the stimulation of myeloperoxidase and phagocytosis activities by A23187. When the enzyme activity was assayed, the cells were incubated without microspheres. At the time indicated, the cells were collected by centrifugation and were extensively washed with PBS. The homogenates of the resulting cells were used as the enzyme source. Phagocytosis was determined as described in Fig. 1.
○, control; ●, 2.5 μM A23187.

Fig. 2 describes time courses of stimulation of phagocytosis and myeloperoxidase activities induced by A23187. Phagocytosis was stimulated by A23187 in the incubation time-dependent manner. Maximum stimulation of phagocytosis by this compound was observed 20 min after the addition of the ionophore. On the other hand, myeloperoxidase activity was the highest 2.5 min after the incubation in the presence of A23187. The activation of the enzyme by the ionophore was gradually decreased 5 min after the incubation. Since myeloperoxidase was excreted into medium when the neutrophils were activated (15), the release of the enzyme into the medium induced by A23187 was also examined. The activity of myeloperoxidase was detected in the medium 10 min after the addition of A23187 (data not shown). Therefore, the decrease in the activation of the enzyme may be due to the release of the enzyme into the medium by A23187. The stimulation of phagocytosis and the enzyme activity by A23187 were also dependent on its concentration (data not shown). Both activities were significantly stimulated by 1 μM A23187. The maximum stimulation was observed at 2-3 μM A23187. The concentration-response curve for activation of myeloperoxidase by A23187 was similar to that of phagocytosis. The stimulatory effect of A23187 was disappeared

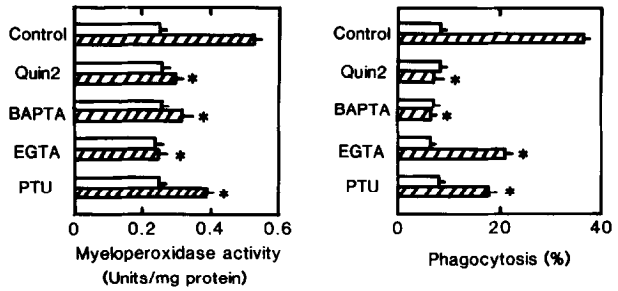

Fig. 3. Effects of various compounds on the stimulation of the activities of myeloperoxidase and phagocytosis induced by A23187. Quin-2 (50 μ M) and BAPTA (30 μ M) were preloaded to the cells as their tetraacetoxymethyl ester at 37° C for 30 min. The cells were extensively washed after the preincubation, and then the activities of phagocytosis and the peroxidase were determined in the presence or absence of 2.5 μ M A23187 as described in Figure 1 and 2. Phagocytosis was expressed as % of total phagocytized cells. EGTA (8 mM) was simultaneously added with A23187. PTU (100 μ M) was preincubated with the cell at 37° C for 20 min and then A23187 was added. PTU, propylthiouracil.
□, control; ▨, 2.5 μ M A23187.
*$P<0.05$, compared with control.

by solubilization of the cell treated with cetyltrimethylammonium bromide. In addition, the purified myeloperoxidase could not be activated by A23187, indicating that this ionophore is likely to act on the intact cells.

To clarify the mechanism of the stimulation by A23187, the effects of various compounds on the stimulation of the activities of phagocytosis and myeloperoxidase induced by A23187 were examined (Fig. 3). Propylthiouracil, a potent inhibitor of myeloperoxidase through the formation of catalytically inactive Compound II in the presence of hydrogen peroxide (17), significantly inhibited not only activation of myeloperoxidase by A23187 but also stimulation of phagocytosis. This evidence supports that stimulation of phagocytosis by A23187 was accompanied by activation of myeloperoxidase. Quin-2 and BAPTA, intracellular Ca^{2+} chelators, prevented the stimulation of both activities induced by A23187 (Fig. 3). The stimulation of both activities were also inhibited by EGTA. On the other hand, these compounds had no effect on the activities of both peroxidase and phagocytosis without A23187. Thus, A23187 is likely to stimulate

the activities of phagocytosis and myeloperoxidase through an increase in intracellular Ca^{2+} concentration.

The results obtained in this experiment show that the activities of phagocytosis and myeloperoxidase in polymorphonuclear leukocytes of rats were activated by A23187. Further, phagocytosis stimulated by A23187 were prevented by the inhibitor of myeloperoxidase. Therefore, activation of myeloperoxidase by A23187 may be involved in the stimulation of phagocytosis. The intracellular Ca^{2+} chelators completely blocked the stimulations of both phagocytosis and myeloperoxidase, indicating that the stimulatory effect of A23187 is due to the increase in intracellular Ca^{2+} concentration. These findings suggest that myeloperoxidase play an important role in phagocytosis of polymorphonuclear leukocytes besides the bactericidal function.

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