The Influence of Some Metabolic Inhibitors on Phagocytic Activity of Mouse Macrophages in vitro

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Summary. The action of different metabolic inhibitors on phagocytosis by macrophages from mouse peritoneal exudate cultured in vitro was studied. The following metabolic inhibitors were tested: sodium iodoacetate, sodium fluoride, sodium fluoroacetate, sodium malonate, 2-4-dinitrophenol, sodium azide, ouabain and cycloheximide, all at the concentration of 10⁻³ M. Iodoacetate caused a strong inhibitory effect on phagocytosis; this observation confirms that glycolysis is the main source of energy for the phagocytic process.

On the contrary, fluoride, although it is an effective inhibitor of glycolysis, did not exert any effect. This difference may be explained by the fact that sodium fluoride blocks anaerobic glycolysis only in vitro at an unphysiological temperature (0°C). Fluoroacetate and malonate, two compounds which interfere with the Krebs cycle, did not inhibit phagocytosis, but it is known that the Krebs cycle activity is poorly developed in the macrophagic cells. Sodium azide and 2-4-dinitrophenol, two inhibitors of oxidative phosphorylation, showed an effect on phagocytosis only after 3 h of contact with the cell cultures. Ouabain blocks Na⁺ and K⁺ transport across the plasma membrane and, probably, it inhibited phagocytosis by interfering with the movements of the cell membrane. Finally, the mode of action of cycloheximide on phagocytosis is uncertain. This compound inhibits the protein synthesis and, perhaps, it can act by preventing the renewal of the cell membrane.

Key words: Phagocytosis – Phagocytic activity – Macrophages – Metabolic inhibitors

The phagocytic process plays an important role in the host defence against the infectious agents and it is accompanied by several biochemical modifications in the phagocytizing cells.

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The energy required by the engulfment of foreign particles appears to come from glycolysis rather than from oxidative phosphorylation, as it is shown by using different metabolic inhibitors. For example, substances blocking anaerobic glycolysis, such as iodoacetate, arsenate and fluoride, markedly decrease the uptake of particles by polymorphonuclear leukocytes, while inhibitors or uncouplers of cytochrome-linked phosphorylation, such as cyanide, antimycin and 2-4-dinitrophenol do not have such an effect [3, 20].

On the contrary, the uptake of foreign particles is followed by a remarkable enhancement of the oxygen consumption in different cell kinds such as human, guinea pig and rabbit alveolar macrophages. In these cells the inhibitors of respiratory chain and a low oxygen tension strongly inhibit the phagocytic activity [27, 6, 22].

These same experimental conditions show only a poor effect on human, guinea pig, rat and rabbit polymorphonuclear leukocytes which derive their energy mainly from glycolysis and possess a rudimentary Krebs cycle activity [23, 4, 21].

On the other hand, pinocytosis, a biological event strongly similar to phagocytosis, is blocked in mouse peritoneal macrophages by some substances, such as cyanide, puromycin and 2-4-dinitrophenol, which act on respiratory chain or on oxidative phosphorylation [9, 10, 12, 8].

In spite of extensive experiments carried out on the influence of various metabolic inhibitors on the phagocytic activity, no clear picture has emerged about these experimental results. This was due mainly to the fact that in vitro assays have been carried out on different cell kinds and using different methods for measuring the phagocytic activity. Most of the experiments, in fact, were carried out on leukocytes suspensions which are readily obtainable from human peripheral blood, but a large variety of experimental models, such as monolayer cell cultures of macrophages from peritoneal exudate or of alveolar origin, derived from mouse, guinea pig and rabbit, was also used.

The present experiments were undertaken to elucidate the metabolic behaviour of mouse peritoneal macrophages during the attachment and the ingestion of zymosan particles by testing the influence of different metabolic inhibitors so far as possible under standardized conditions.

Material and Methods

Animals

In all experiments 8 weeks old male albino mice (Swiss strain) weighing about 40 g were used. The animals were fed on a synthetic diet.

Cell Cultures

A macrophage suspension was obtained by injecting 2ml of a starch suspension in sterile distilled water (1.5 g/100 ml) into the peritoneal cavity of the mice. Two days later, the animals were killed by cervical dislocation and the cells were washed out from the peritoneum with 2 ml of culture medium. The cells were centrifuged at about 50 g for 5 minutes in a refrigerate centrifuge and resuspended in Parker's medium 199 without bicarbonate, dried (Difco) supplemented with 15% of fetal calf serum (Microbiological Associates) and with 10% of lactalbumin.