Macrophage Cultures: An Extracellular Esterase

Abstract. *An enzyme having esterase activity accumulates in the culture media of mouse peritoneal mononuclear leukocytes during differentiation of the cells into macrophages. It has a* p*H optimum of 6.5 and shows aryl esterase characteristics. The esterase differs from another macrophage hydrolase, acid phosphatase, in its mainly extracellular distribution.*

The major known functions of macrophages are ingestion of microorganisms and other colloidal particles and subsequent degradation of the engulfed material by lysosomal hydrolases (*1*). One may follow the synthesis of these hydrolytic enzymes during the differentiation in vitro of mouse peritoneal mononuclear leukocytes into macrophages (*2, 3*).

In the course of this differentiation process there is a 5- to 15-fold increase in the culture content of intracellular acid hydrolases, such as acid phosphatase, β-glucuronidase, cathepsin D, and acid ribonuclease. This report deals with an acid esterase that accumulates in the extracellular fluid of cultures of mouse peritoneal mononuclear leukocytes during their differentiation into macrophages.

Mononuclear leukocytes were collected from the peritonea of mice and cultured in a manner described (*3*). Cultures were harvested after 2 hours and 1, 2, 3, and 4 days, and the media were centrifuged at low speed for removal of any detached cells. Lysates of the cell monolayers were prepared in a manner described (*3*). The cell lysates and the culture media were assayed for nonspecific esterase at *p*H 6.5 with β-naphtyl acetate as a substrate (*4*), and for acid phosphatase (*3*). When media from cell cultures were assayed for enzymic activity, sterile medium that had not been in contact with cells served as a blank. The results are summarized in Fig. 1.

The total esterase activity of the mononuclear leukocyte cultures, calculated as the sum of the extra- and intracellular activities, is low during the first day but increases with the maturation of the cells into macrophages. When examined on the fourth day it shows a 100-fold increase over the 2-hour value.

This increase in esterase activity exceeds the rise in activity of total acid phosphatase during the differentiation process. Furthermore, there is a marked difference between the intra- and extracellular distributions of the two enzymes: most of the esterase is recovered from the medium, while the acid phosphatase is mainly intracellular. During the third and fourth days, when the cells show typical macrophage features (*2, 3*), the ratio between extra- and intracellular esterase reaches values of 13 and 27, respectively, as compared to 0.3 and 0.8 for acid phosphatase.

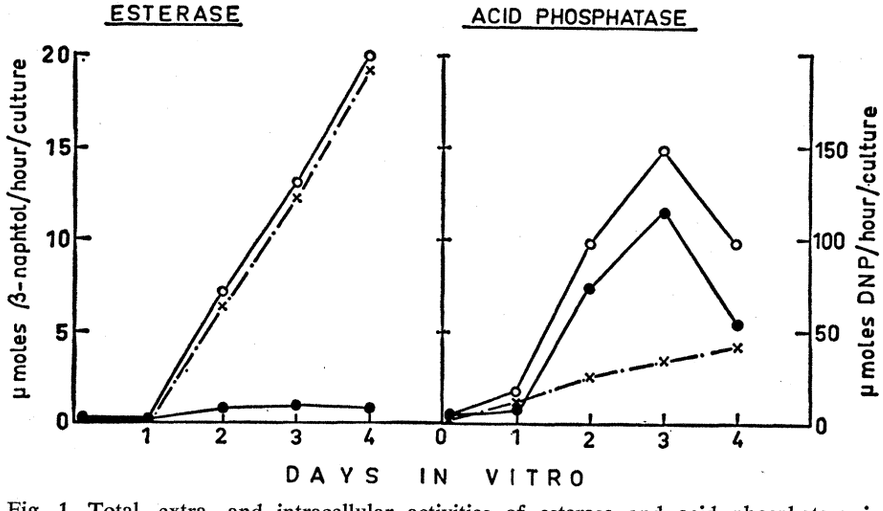

Fig. 1. Total, extra- and intracellular activities of esterase and acid phosphatase in cultures of mouse peritoneal mononuclear leukocytes during their differentiation into macrophages. Symbols: ○——○, total enzyme activity; ×—·—·×, extracellular enzyme activity; ●——●, intracellular enzyme activity.

The esterase activity of the culture medium had a *p*H optimum of 6.5. While the enzyme is active toward β-naphtyl acetate, it does not decompose β-naphtyl laurate. *p*-Chloromercuribenzoate, in a concentration of $10^{-4}M$, gives 10- to 20-percent inhibition of the esterase, which is not affected by $10^{-4}M$ diisopropyl fluorophosphate.

On the basis of the resistance to diisopropyl fluorophosphate and the slight inhibition by *p*-chloromercuribenzoate, the extracellular macrophage esterase falls most closely into the group of aryl esterases (*5*). Its acid *p*H optimum and its resistance to an organophosphorous inhibitor of esterases resemble the behavior of the lysosomal esterase found in rat liver (*6*).

The mainly extracellular location of the macrophage esterase activity may be related to Holt's observation that lysosomal esterase activity from frozen dried tissue sections can be easily leached by $0.25M$ sucrose, a treatment that does not wash out acid phosphatase activity (*7*). This finding, together with our observation, suggests an esterase that is loosely attached to the outer surface of the lysosomal membrane (*7*) before it reaches the extracellular medium.

It is still unclear whether or not the extracellular macrophage enzyme possesses additional hydrolytic activities as have been attributed to other esterases (*8*); elucidation of this question and further characterization of the esterase await its purification.

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17 August 1967