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Various sensitivity of macrophage enzymes for endogenous proteases

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It is generally accepted that the intracellular proteins are in a dynamyc state due to its continual degradation and synthesis. These are several pathways of breakdown of the intracellular protein. Ubiquitin, a small protein found in most eucaryotic cells which is a marker for rapid degradation of the abnormal, normal, and short-lived proteins [7]. It is noted that the activation of the ubiquitin is an ATP-requiring process. Recent studies indicate that multiple signals exist within protein substrates which plays a decision role in determining of the way of proteolysis and their intracellular halflife. An other way the intracellular degradation of protein and many membrane proteins carried out by lysosomes. The lysosomal pathways of protein degradation are most prominent condition such as starvation of the organisms or any pathological conditions.

The macroautophagic vacuoles are believed to be formed in situ from membranes derived from the endoplasmic reticulum [2]. The macroautophagic vacuoles are formed from Golgy apparatus and smooth endoplasmic reticulum subsequently fuse with primary lysosomes. It is well known that lysosomal proteolysis is highly regulated process and depends on the cell type and assay conditions.

In this paper reported here we investigated the effect of the leucine-O-methyl ester (Leu-OMe) as a lysosomotropic agent on protein metabolism and on the activity of some enzymes: non specific esterase, acid phosphatase, peroxidase, lysoz yme, arginase, and lactat dehidrogenase of the mouse peritoneal exudate cell (PEC).

Materials and Methods

CFLP male mice (25–30 g, LATI, Gödöllő, Hungary) were injected i.p. with 1.0 ml of 2% caseine (Hammarsten Merck), and 72 hours later the elicited cells were collected by washing the peritoneal cavity with 5.0 ml of Ca^{2+}, Mg^{2+} free PBS. PEC (3×10^6) were attached for 1 hr to the bottom of plastic tissue culture dishes, at 37 °C in a humidified atmosphere of 5% CO_2 and air in ASSAB CO_2 incubator. The adherent macrophages (MØ) were treated subsequently with 5 mmole of Leu-OMe for 60 mins at 37 °C. The MØ then were washed three times with PBS. Incorporation of L-/U-^{14}C/-valine (469 MBq/mmole) into protein was expressed as a cpm/μg protein. Activity of non specific esterase [5], lysozyme [6], lactate dehydrogenase

[8], acid phosphatase [9], arginase [10], and myeloperoxydase [11] enzyme activities were monitored spectrophotometrically.

Results and Discussion

According to our previous electronmicroscopic ultrastructural studies it was observed the dilatation and large amount of vacuola formation associated with 5 mmole Leu-OMe treatment of the freshly harvested PEC [3, 4]. We found that the phagocytic capacity of the treated cells is seriously impaired and the incorporation of ^{14}C-amino acids – the synthesis of the protein is abolished [1].

Some qualitative histochemical investigations proved to be the decrease the myeloperoxidase, acid phosphatase within the cells treated with Leu--OMe [4]. These observations indicated the investigation of some other enzymes (secreted and non secreted) of the PEC. The increasing LDH activity was showed out in extracellular media indicating the changes of treated cell permeability with Leu-OMe. The decrease of the secretion of arginase and decrease of the nonspecific esterase activity and increase of secretion of acid phosphatase were observed. We conclude from results obtained that Leu-OMe causes a disruption of lysosomes which is lead to impair or abolish of the function of endoplasmic reticulum because of its high sensitivity of the lysosomal enzymes. Furthermore some of endogenous protease will be activated and some of the intracellular enzymes splitting depends on their amino acids or on their topographical location within the MØ. Although numerous investigations carried out concerning to the effect of the Leu-OMe the detailed mechanism of the process is still poorly understood However the underlying biochemical processes remain to be identified.

References

1. Antoni F, Csuka I, Hrabák A, Temesi A, Szende P, Lapis K: The effect of L-Leucine methyl ester on the amino acid incorporation of murine peritoneal cells: Int. 3. Immunopharmatology in press 1989
2. Arstila AU, Trump BF: Studies on cellular autophagocytosis. American J Pathology 53: 887, 1968
3. Csuka I, Szende B, Antoni F, K. Lapis: Effect of O-methyl esters of amino acids on macrophages. Proc. Sec. Int. Conf. on the role of formaldehyde in biological systems. SOTE press 27, 1987
4. Csuka I, Szende B, Antoni F, Lapis K: Specific effect of leucine-O-methyl ester on macrophages. Acta Paediatr Hung 1988. (in this issue)
5. Frackowiak J, Szutowicz A: Acid α-naphthyl acetate esterase assay in murine lymphocytes. Analytical Biochemistry 165: 269, 1987
6. Gordon S, Todd J, Cohn ZA: In vitro synthesis and secretion of lysosyme by mononuclear phagocytes. J Exp Med 139, 1228, 1974
7. Hershko A, Ciechanover A: The ubiquitin pathway for the degradation of intracellular proteins. Progress in Nucleic Acid Research. Molecular Biology 33: 19, 1986
8. McMillan RM, Macintyre DE, Beesley JE, Gordon JL: Regulation of macrophage lysosomal enzyme secretion: role of arachidonate metabolites divalent cations and cyclic AMP. J Cell Sci 44: 299, 1980
9. Saito K, Suter E: Lysosomal acid hydrolases in mice infected with BCG. J Experimental Medicine 121: 727, 1965
10. Schimke RT: Methods in enzymology ed. Colowick SP and Kaplan NO 17: 313, Academic Press, New York 1970
11. Yamada M, Mori M, Sugimura T: Purification and characterization of small molecular weight myeloperoxidase from human promyelocytic leukemia HL-60 cells. Biochemistry 20: 766, 1981