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ADAPTED MICROASSAY FOR PHAGOCYTIC ABILITY OF HUMAN BLOOD MONOCYTES

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ABSTRACT

The aim of this study was to obtain a pure monocyte population and to examine the phagocytic and bactericidic ability of these cells by using our micromethod for in-vitro assay. We worked out a relatively simple method for examination of the phagocytic ability of monocytes including production of pure monocytes, its adhesion on coverslips and cultivation with autologous or AB-serum and test-bacteria in microcamera constructed by us.

Key words: monocytes, phagocytosis, index of phagocytosis, phagocytic number, index of bactericidity

INTRODUCTION

Phagocytosis can be mediated by a variety of cells associated with the blood system. The two major categories of cells participating in this process are the polynuclear phagocytes (granolucytes - Gr) and mononuclear phagocytes (monocytes - Mo and macrophages - Ma) (9, 17, 18, 21).
Morphologic and cytochemical studies indicate that monocytes isolated from mammalian peripheral blood can differentiate into large cells resembling tissue Ma (14). Such phagocytic mononuclear cells including the blood monocytes may have a defense functions against microorganisms (18, 19).
The use of pure cell populations is required for the diagnosis of impaired phagocytic ability. Pure monocytes can be obtained by methods based on the differences in cell density (6, 7, 8) and in adhesion ability, too (10, 11).
The aim of this study was to obtain a pure monocyte population and to examine the phagocytic and bactericidic ability of these cells by using our micromethod for in-vitro assay.

MATERIAL AND METHODS

Monocytes were isolated from 8 ml heparinized blood derived from 8 healthy persons. The blood was diluted with saline buffer (16). In siliconizided tubes density gradient was prepared composed of 2 separation media (A and B) and characterized by us according to the data of Eckert (12).

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Medium A, with density of 1,1g/ml at 18oC was composed of 13 parts 34% Uropolin and 24 parts 14,6% Ficoll-400. Medium B with density of 1,75g/ml at 18°C was composed of 10 parts 34% Uropolin and 24 parts 9% Ficoll-400.
In the tube, medium A, B and diluted blood were layed in consequence. The samples were centrifuged at 400xg for 40min at 22°C.
Three cell fractions were formed: fraction I - at the bottom of the tube (erythrocytes); fraction II - between medium A and B (granulocytes), and fraction III - above medium B (monocytes and lymphocytes).
From fractions II and III smears were prepared and the cells were identified by the cytochemical method of Jam (13). Fraction II and III were derived using the devices constructed by us (3, 4). The cells were triply washed with cold saline and suspended in Hank's balanced salt solution containing 20% autologous serum. Then 0,5ml of fraction III were applied on a coverslips (24x32mm, Germany), mounted in the microcamera for microcultures constructed by us on coverslips (1). The microcamera was placed at 37oC, 7,.5% CO_2. The cells (Mo and Ly) were allowed 60min to adhere on the coverslips. The nonadherent cells (lymphocytes) were washed out with Hank's balanced solution. The adherent cells were applied with 0,3ml TC-199 containing 20% autologous or AB-human serum and 10x104 Staphylococcus aureus/ml (ATCC 25923) (standardized suspension of 24-hours culture). After 15-min intervals the adhered cells on the coverslip from each individual were stained with acridine orange. The coverslips were mounted on a slide and the cells were analyzed by ML-2A luminescent microscope (2). One-hundred cells from each preparation were counted and quantitatively characterized by index of phagocytosis (Iph) = % of phagocyted cells; phagocytic number (PhN) = number of phagocyted bacteria/number of phagocyted cells, and index of bactericidity = % of the killed phagocyted bacteria (5).

The vitality of the adhered cells and bacteria was determined by the method of Smith and Rommel (20).

RESULTS AND DISCUSSION

Our results indicate that the cell composition of the separated fractions was 98-99% granulocytes in fraction II, 78-84% lymphocytes and 16-22% monocytes in fraction III. It is possible to obtain pure granulocyte population and mononuclear cells enriched with monocytes under the above described experimental conditions. The purity of the granulocyte fraction is very close to the data (85%-90%) presented in our previous publication (2).

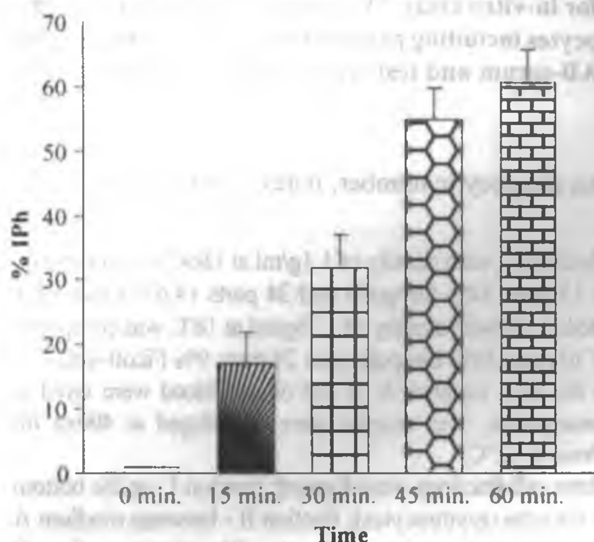

Fig. 1. Kinetics of index of phagocytosis (IPh) of macrophages obtained from human blood ($p_{15-30} < 0,05$; $p_{30-45} < 0,001$; $p_{45-60} < 0,01$)

As the monocytes are heterogenic cells with overlapping densities, the obtaining of pure monocyte population by density-gradient centrifugation is relatively difficult (15).

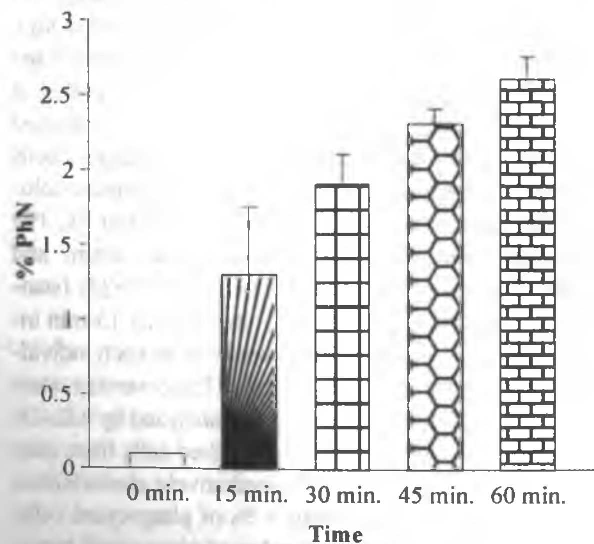

Fig. 2. Phagocytic number (PhN) of macrophages obtained from human blood ($p_{15-30} < 0,01$; $p_{30-45} < 0,05$; $p_{45-60} < 0,01$)

For that reason, fraction III enriched with monocytes was additionally purified according to the adhesion properties of these cells.

The thin layer of cells formed on the coverslips contains $2x10^3$-$5x10^3$ monocytes per coverslip with purity of 94-98%.

The most suitable concentration of Staphylococcus aureus for the assay of the phagocytic ability of the adhered cells was $10x10^4$ bacteria/ml.

It can be seen from Fig. 1 that IPh is highly elevated to the 45th min. At the 60th min the elevation is lower, but significant.

The PhN and the IB (Fig. 2 and Fig. 3) were highly elevated to the 30th min. The values obtained at the 45th and 60th min were significantly different only for PhN. It can be supposed that some of the phagocyted bacteria are ingested and therefore it is difficult to identify them (2, 20).

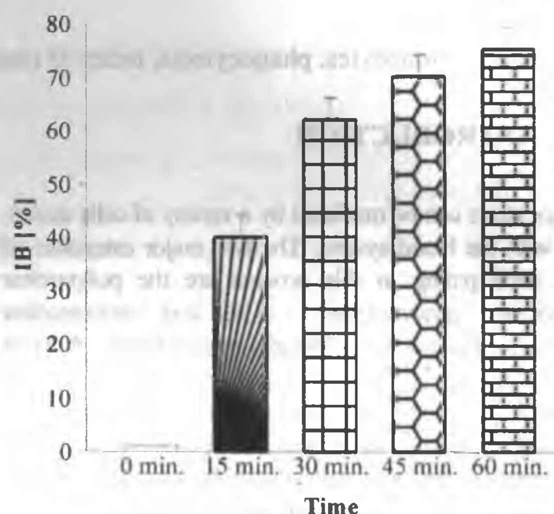

Fig. 3. Index of bactericidity (IB) of macrophages obtained from blood ($p_{15-30} < 0,001$; $p_{30-45} < 0,05$; $p_{45-60} > 0,01$)

Having in mind the changes in IPh and PhN and the fact that the values in IB for 45th and 60th min are not significant we conclude that the 45-min incubation is the most appropriate for examination of the phagocytic ability of monocytes. In a previous study we found that the same time of incubation (45 min) is suitable for granulocytes, too (2).

Our previous investigations on IPh of adhered cells with different concentrations (10%, 20%, and 30%) of autologous serum indicate that the most appropriate concentration was 20%. It can be seen from Table 1 that the values of IPh obtained by the use of autologous or AB-serum are insignificantly different, so both sera are suitable for examining the phagocytic ability of monocytes. The percent of phagocyted monocytes is lower than that of phagocyted granulocytes and the percent of the monocytes ingested more than 10 bacteria is also lower than that of granulocytes. That is why we conclude that the monocytes are least effective phagocytes of bacteria under our experimental conditions.

The enrichment of mononuclear cells with monocytes, its adhesion on coverslips and cultivation in our camera for

Table 1. Comparative examination of IPh and percentage of phagocytes ingested more than 10 bacteria after 45-min incubation with Staphylococcus aureus

Cells	Medium	IPh	% of cells ingested > 10 bacteria
1. monocytes	TC 199 + 20% autologus serum	59,2 ±6,5	1,9 ±1,0
2. monocytes	TC 199 + 20% AB-serum	54,5 ±7,8	2,2 ±0,9
3. granulocytes (fraction II)	TC 199 + 20% autologus serum	68,1 ±6,0	3,9 ±0,9
4. granulocytes (nonheparinized blood)	TC 199 + 20% autologus serum	76,8 ±7,6	5,1 ±1,4

IPh: p 1-2 > 0,01; p 1-3 < 0,05; p 3-4 > 0,01 % of cells ingested > 10 bacteria: p 1-3 < 0,01; p 3-4 > 0,01

microcultures with autologous or AB-serum and test-bacteria are relatively simple methods for examination of the phagocytic ability of monocytes.

REFERENCES

1. Диванян, Х., Д. Цанев, Л. Франке. Ротатор на култури в епруветкн и микрокултури върху покривни стъкла. Рег. № 195/86 на ТИС при МА-София.
2. Диванян, Х., Д. Цанев, Л. Франке, К. Божкова. In vitro микрометод за изследване на фагоцитарната и бактерицидна активност на гранулоцити от периферна кръв. - *Експерим. мед. морфол.*, **28**, 1989, 49.
3. Цанев, Д., Х. Диванян. Глава на перисталтична помпа с приспособление за компенсиране на пулсациите. Рег. № 252/87 на ТИС при МА-София.
4. Цанев, Д., Х. Диванян. Прибор за изливане на градиенти и за извличане на фракции. Рег. № 117/87 на ТИС при МА-София.
5. Меньшикон, Б. Лабораторные методы исследования в клинике. Справочник. Москва, Медицина, 1987, 364 с.
6. Baran, J., E. Pajdak, M. Isendorf, D. Szmyd. Evaluation of the interactions between strains of S. aureus and peripheral blood phagocytic cells.- *Med. Dosw. Mikrobiol.*, **50**, 1998, 131-140.
7. Baran J, E. Pajdak, M. Isendorf, D. Szmyd. Evaluation of interactions between strains of S. aureus isolated from different clinical specimens with peripheral blood phagocytic cells.- *Med. Dosw. Mikrobiol.*, **51**, 1999, 37-46.
8. Brodersen, M., P. Burns. The separation of human monocytes from blood including biochemical observation.- *Proc. Soc. Exp. Biol. Med.*, **144**, 1973, 941.
9. Clark, W. The experimental foudations of the modern immunology, New York, John Willey and Sons, 1980, 372.
10. Cline, M., R. Lehrer. Phagocytosis by human monocytes.- *Blood*, **32**, 1968, 423.
11. Dekaris, D. Purification of monocytes.- *Clin. Exp. Immunol.*, **16**, 1974, 311.
12. Eckert, R. Immunzellen.- In: Immunologische Arbeitsmethoden. Jena, VEB Gustav Fischer Verlag, 1984, 253-281.
13. Jam, L., C. Li, W. Krosby. Cytochemical identifications of monocytes and granulocytes.- *Amer. J. Clin. Pathol.*, **55**, 1971, 283.
14. Kusner, D., J. Adams. ATP-induced killing of virulent Mycobacterium tuberculosis within human macrophages requires phospholipase.- *J. Immunol.*, **164**, 2000, 379-88.
15. Loos, H., B. Blok-Shut, R. van Doom, et al. A method for the recognition and separation of human blood monocytes on density gradients.- *Blood*, **48**, 1976, 731.
16. Marodi, L, P. Leijh, R. van Furth. A micromethod for the separate evaluation of phagocytosis and intracellular killing of Staphylococcus aureus by human monocytes and granulocytes.- *J. Immunol. Methods*, **57**, 1983, 353-561.
17. Raybourne, R., G. Roth, P. Deuster, E. Sternberg, A. Singh. Uptake and killing of Listeria monocytogenes by normal human peripheral blood granulocytes and monocytes as measured by flow cytometry and cell sorting.- *FEMS Immunol. Med. Microbiol.*, **31**, 2001, 219-225.
18. Stuart, A., J. Habeshaw, A. Davidson. Phagocytes in vitro.- In: Handbook of Experimental Immunology. Vol. 2. Cellular Immunology. D. Weir, ed. Oxford, Blackwell Scientific Publications, 1980, 31.1-31.30.
19. Suter, E. Multiplication of tuberculous bacilli within mononuclear phagocytes in tissue cultures derived from normal animals and animals vaccinated with BCG.- *J. Exp. Med.*, **97**, 1953, 235.
20. Smith, D., F. Rommel. Vitality of phagocyted cells and bacteria.- *J. Immunol. Methods*, **17**, 1977, 241.
21. R. van Furth, L. Theda, van Zwet, C. Leijh. In vitro determination of phagocytosis and intracellular killing by polymorphonuclear and mononuclear phagocytes.- In: Handbook of Experimental Immunology. Vol. 2. Cellular Immunology. D. Weir, ed. Oxford, Blackwell Scientific Publications, 1980, 32.1-32.19.