Blut (1981) 42: 379–382

Blut
© Springer-Verlag 1981

Short Communication / Kurze Mitteilung

Flow-cytometric Measurements of the Transmembrane Potential, the Surface Charge Density and the Phagocytic Activity of Guinea Pig Macrophages After Incubation with Lymphokines

G. Valet[1], H. L. Jenssen[2], M. Krefft[1], and G. Ruhenstroth-Bauer[1]

[1] Max-Planck-Institut für Biochemie, Abteilung für Experimentelle Medizin, D-8033 Martinsried, Federal Republic of Germany
[2] Arbeitsgruppe für Tumorimmunologie, Bereich Medizin, Wilhelm-Pieck-Universität, DDR-25 Rostock, German Democratic Republic

Summary. The effects of lymphokines on guinea pig peritoneal macrophages were measured via flow cytometry utilizing the three-parameter FLUVO-METRICELL flow cytometer. On the basis of cell volume three distinct macrophage populations could be distinguished. Three to 5 min after starting the incubation with lymphokines a hyperpolarization of all three macrophage populations took place which was followed by depolarization. After 60 min the transmembrane potential reached again its control values. The negative charge density of the cell membrane decreased shortly after beginning of the incubation to 70–80% of the initial value and then remained unchanged for the following 120 min. The phagocytic activity of the macrophages was diminished during the depolarisation phase but increased over control values after restoration of the transmembrane potential.

Key words: Macrophage – Lymphokine – Transmembrane potential – Phagocytosis – Electrophoretic mobility – Flow cytometry

Introduction

Lymphokines have an important function as signal substances of stimulated lymphocytes. They influence several macrophage properties, such as the electrophoretic mobility [2,4], the velocity of locomotion (migration inhibition factor [MIF]) [1], the transmembrane potential [3], and to some degree the phagocytosis and intracellular enzymatic activity [7]. The experimental determination of lymphokine effects on

---

Offprint requests to: G. Valet, MD (address see above)

0006-5242/81/0042/0379/$ 1.00

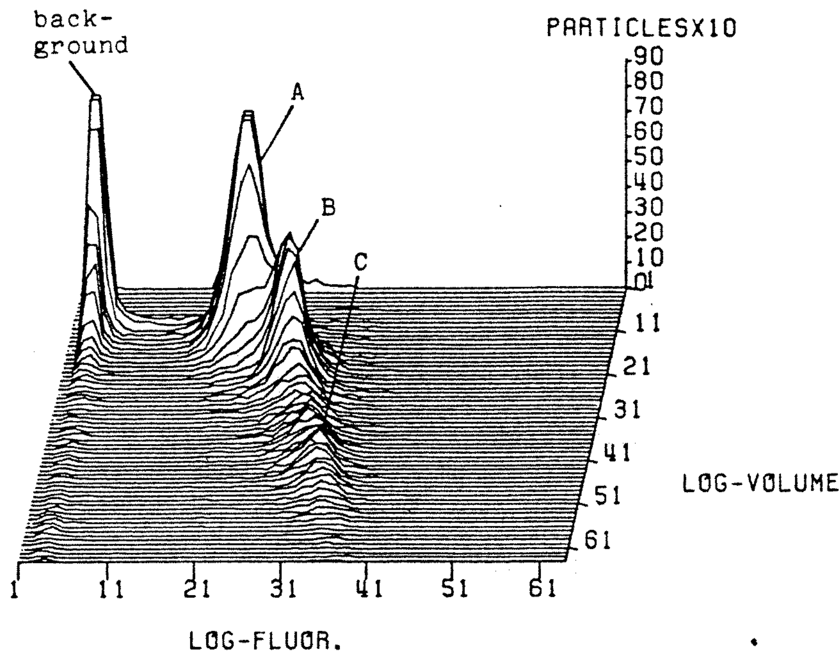

Fig. 1. Simultaneous measurement of cell volume and fluorescence of the transmembrane potential sensitive dye DIOC6 in guinea pig peritoneal macrophages. The macrophages were taken from the peritoneal cavity 6 days after injection of paraffin oil. Three distinct macrophage populations (ABC) can be distinguished by cell volume. The logarithmic scales comprise 2.5 decades

macrophages is usually done separately for each parameter. The procedures are time-consuming, and a simultaneous determination of several properties of the same cell is often not possible even in principle. Such measurements are, however, important since macrophages are a heterogeneous cell population with regard to their state of stimulation or activation. It is possible that macrophage subpopulations react differently to the same external stimuli.

The aim of this investigation was to accelerate measurements of macrophage parameters by the use of a flow cytometer, and additionally to exploit the capability of such instruments to determine two or three parameters of an individual cell simultaneously [5,9].

Methods

We have measured the cell volume of paraffin oil-induced guinea pig peritoneal macrophages by electrical sizing in a hydrodynamically focused orifice [6], the transmembrane potential with the membrane potential sensitive dye, 3,3-dihexyloxacarbocyanin (DIOC6) [8], the phagocytosis by ingestion of rhodamin stained, monosized latex particles of 1.8 μm diameter, and the negative surface charge density of the cell membrane, which is a measure for the electrophoretic mobility of cells, by staining with fluorescinated poly-l-ornithine [10]. The macrophages were incubated prior to the measurement at 37°C with lymphokine-containing supernatant obtained from either nonspecifically ConA-stimulated mouse spleen cells or from PPD-tuberculin restimulated guinea pig lymphocytes from an animal which had previously been immunized by complete Freund's adjuvant.

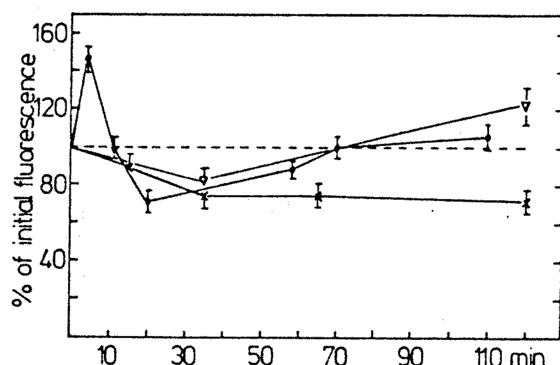

Fig. 2. Changes of the transmembrane potential (●), of the negative surface charge density (×) and of the phagocytic activity (▽) of guinea pig macrophages after incubation with lymphokine containing supernatant from sepharose-ConA stimulated mouse spleen cells. The three macrophage populations behaved similarly with regard to the relative changes of their parameters. The mean and the standard deviations in this graph were calculated from the per cent of initial values of the three macrophage populations. Each mean value corresponds to the measurement of between $0.8-1.0 \times 10^5$ single cells

Results

Three discrete macrophage subpopulations could be distinguished by the cell volume measurement alone (Fig. 1). Two to 5 min after incubation with lymphokine containing supernatant a hyperpolarization of the transmembrane potential of all macrophages was observed (Fig. 2), followed by a depolarization below control values until 20 min after the start of the incubation. A slow repolarization occurred up to 60 min when the control values were reached again. The negative charge density (Fig. 2) of the cell membrane decreased during the depolarization phase to between 70 and 80% of the initial values. It remained decreased until the end of the experiment after 120 min. The phagocytosis of latex particles (Fig. 2) was decreased during the time of depolarization. After repolarization it was, however, increased. The speed of phagocytosis of the three macrophage population was different. Although the large macrophages phagocytosed a greater quantity of particles than the small macrophages, it was interesting that the small macrophages were more active if the phagocytosis was calculated per unit cell volume or unit cell surface area. Phagocytosis was neither directly proportional to the cell surface nor to the cell volume, suggesting that the three macrophages were different in their functional activity.

Discussion

The flow cytometric results confirm and extend earlier observations of Jenssen et al. [3] who demonstrated by microelectrophoresis measurement and by transmembrane potential measurements with intracellular electrodes that a decrease of the electrophoretic mobility and of the transmembrane potential of guinea pig macrophages occurs after incubation with lymphokine containing supernatants. The pre-

sent results show that flow cytometry offers the important advantages of speed and simultaneous measurement over the classical methodology. It is also important that no preparative separation or enrichment of the macrophages is necessary prior to the flow cytometric measurement because macrophages can be well dinstinguished from smaller lymphocytes by a simultaneous volume measurement. Since the cells can be measured shortly after aspiration from the peritoneal cavity the possibilities for changes of the cell parameters are minimal. The staining methods in this investigation were vital stains which are complete within 2–10 min. It is not necessary to remove remaining unbound dye by washing of the cells which means that the fluorescence measurement can be performed under defined biochemical equilibrium conditions. Multiparameter flow cytometry is therefore a useful tool for the investigation of complex interactions in the immune effector system. It overcomes the lack of easily and precisely quantifiable reactions which often is a problem in the analysis of cell mediated immune reactions. Our results offer also the possibility to develop routine test procedures for clinical laboratories (e.g., macrophage electrophoresis mobility test [MEM] for the early detection of tumor disease in man) since flow cytometers are capable of measuring in the order of 10^5 cells/min. This will improve the statistical reliability of the tests which, together with the biological variability, is frequently a problem when using traditional methods for determination of cell electrophoretic mobility.

References

1. Block LH, Jaksche H, Bamberger S, Ruhenstroth-Bauer G (1978) Human migration inhibitory factor: Purification and immunochemical characterization. J Exp Med 147: 541–553
2. Block LH, Ruhenstroth-Bauer G (1979) Biological and clinical relevance of human macrophage migration inhibitory factor (MIF). Blut 38: 93–101
3. Jenssen HL, Redmann K, Köhler HJ (1975) The effect of a mediator of cellular immunity on the transmembrane potential of macrophages. Acta Biol Med Ger 34: 1907–1910
4. Jenssen HL, Werner H, Köhler H (1976) Lymphokines which influence electrophoretic mobility of macrophages. Acta Biol Med Ger 35: 1499–1503
5. Kachel V, Glossner E, Kordwig E, Ruhenstroth-Bauer G (1977) FLUVO-METRICELL, a combined cell volume and cell fluorescence analyzer. J Histochem Cytochem 25: 804–812
6. Kachel V (1976) Basic principles of electrical sizing of cells and particles and their realization in the new instrument METRICELL. J Histochem Cytochem 24: 211–230
7. Pick E (1979) Mechanism of action of migration inhibitory lymphokines. In: Cohen S, Pick E, Oppenheim JJ (eds) Biology of the lymphokines. Academic Press, New York, 60–119
8. Shapiro HM, Natale PJ, Kamentsky LA (1979) Estimation of membrane potentials of individual lymphocytes by flow cytometry. Proc Natl Acad Sci USA 76: 5728–5730
9. Valet G (1980) Graphical representation of three-parameter flow cytometer histograms by a newly developed FORTRAN IV computer program. Flow cytometry IV. Universitesforlaget, Oslo, p 108–112
10. Valet G, Bamberger S, Hofmann H, Schindler R, Ruhenstroth-Bauer G (1979) Flow cytometry as a new method for the measurement of electrophoretic mobility of erythrocytes using membrane charge staining by fluorescinated polycations. J Histochem Cytochem 27: 342–349

Received October 20, 1980/Accepted January 15, 1981