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LASER FLOW CYTOMETRY: PHYSICAL BASIS AND ITS APPLICATION IN THE PHAGOCYTIC PROCESS ASSESSMENT

This article reviews the physical principles of the laser flow cytometry method, and shows its possibilities for medical and biological science. The method is effective to solve important tasks in the phagocytic process assessment. It is significant to study phagocytic parameters for the comprehensive analysis of diagnosis, either for primary or secondary immunodeficiency conditions – frequent recurrent inflammations, predisposition to postoperative complications.

Keywords: *flow cytometry, neutrophilis, phagocytosis, reactive oxygen species.*

Introduction

Flow cytometry is an effective approach to solve many important issues arising in medicine, cell biology and cell engineering. The flow cytometry method is used for examination of the dispersed media in the mode of item-by-item analysis of the disperse phase elements by light scatter and fluorescence signals. This method is based on the following: a hydrofocusing system is used, which ensures that one cell passes through the flow; the cells are irradiated with the beam of laser; light scatter and fluorescence signals are registered from each cell in cell suspension with velocity up to 3000 cells per second [1].

Suspension of the cells which are pre-stained with fluorescent dyes (monoclonal antibodies conjugated with fluorescent labels are generally used as such agents) is blown under pressure through the capillary. The cells, picked up by the fluid flow, are arranging in turn one after the other, forming such a «chain» – hydrodynamic focusing principle, whereby the laminar flow conditions are provided, non-mixing the suspended cells with surrounding liquid. When the stream crosses the focused laser beam, usually only one cell being detected at once at the point of the stream and beam crossing, thus avoiding artifacts associated with different remoteness of the cells from the point of intersection of the laser beam with the flow. Highly sensitive sensors located closely to the flow, register the light scattering angle of 2 to 19 °, called forward, or small-angle light scatter (FSC), and sidelight scatter (SSC) at 90 °. At the same time the fluorescent labels (FL1, FL2, etc.) irradiation is registered, which has a certain wavelength for each fluorochrome.

Immune cytofluorometric cell analysis is performed by the following parameters:

• FSC (forward side scatter) is the parameter of forward light dispersion, which characterizes the size of cells;

• SSC (side scatter) is the parameter of side light scattering that reflects optical heterogeneity of cell cytoplasm, specific cellular inclusions and cellular granularity. The term «granularity», representing SSC parameter, has a specific cytofluorometricmeaning, and doesn't reflect a morphological concept of «granularity». This parameter used allows to determine the size ratio of the nucleus and the cytoplasm;

• FL1, FL2 – channels for detecting specific signal of fluorescent dye at different wavelengths. The analysis of the information obtained through the light scatter channels allows to specify the peripheral blood leukocytes into three popula-

tions – lymphocytes, monocytes and granulocytes. Lymphocytes are characterized by the smallest size, granulocytesare the largest cells, monocytesare intermediate by the FSC parameters. Lymphocytes show the lowest SSC indices, monocytes– intermediate, and granulocytes – high.

Several fluorescent labels applied allow to realize simultaneous two–, three–color analysis, since each fluorochrome, when passing through the laser beam, emits light of different wavelengths. The most commonly used fluorescein isothiocyanate (FITC) detected by the FL-1 detector (the first fluorescence channel) (green spectrum), the phycoerythrin (PE), the FL-2 detector (yellow spectrum), the less often the tandem cyanine-5 / phycoerythrinand pyridine chlorophyll (PerCp, Cy5/ PE) – FL-3 detector and allophycocyanin (APC) – FL-4 detector. Current models of the devices can be equipped with two or more lasers and four or more photomultipliers, that allows to register various fluorochromes at different wavelengths. When choosing combinations of fluorochromes for simultaneous determination of several cell markers, it is necessary to consider the wavelength of the light source and the ability of the optical system of the device to specify and record the signals from the used fluorochromes simultaneously. The basic model of cytometer containsblue (488 nm) argon air-cooled laser as a light source. Perfect stability and homogeneity of the gas laser beam provides high class measurements. When the output power of the laser is 20 mW, the sensitivity of the measurements is sufficient to study even the poorly expressed cell markers.

Cell biology developed along with the improvement of analytical basis for the quantitative research and methodology of the study of cells and cellular organelles. Development stages included optimization of microspectrometry, utoradiographymethods, and more recently – laser flow cytometry. Phagocytosis system can be defined as a set of cells and humoral factors which provide the basal level of structural homeostasis [2–5]. Phagocytes are the center of phagocytosis. This term reflects one of the most outstanding functional features of these cells, which is the ability to absorb and destroy foreign material. The ultimate variant of any immune response is the destruction of pathogenic or defective structures, and their elimination.

The main mechanisms of destruction in humoral and cellular variants of immune response are associated with phagocytosis. Phagocyte function failure can significantly impair the efficiency and adequacy of any variant of the immune response. Being a highly sensitive indicator both of norm and pathology, phagocytes serve as a useful tool either for immunological or general clinical diagnosis. Study of phagocytic indices is important in comprehensive analysis of diagnosis of primary immunodeficiency states such as chronic granulomatous disease, Chediak–Higashi syndrome, myeloperoxidase, glucose–6–phosphate dehydrogenase deficiency, «lazy leukocytes» syndrome, disorders of serum opsonic activity (complement component deficiency, hypogammaglobulinemia), and secondary immunodeficiency conditions – frequent recurrent inflammatory processes, predisposition to postoperative complications [6–13]. But at the same time, methodological approaches to the assessment of phagocytosis are quite labor-consuming and long-lasting, that prevents their wide application in clinical laboratory practice.

Flow laser cytometry is a modern approach to examination of the structure and function of cells, and its possibilities allow to study the stages of phagocytosis, as well. Analysis of phagocytosis with automatic laser flow cytometryis characterized by high accuracy, objectivity, rapid analysis, easy set up of reactions.

Phagocytic activity of neutrophils was estimated by flow cytometry [14]. The required volume of cells was added to the flow cytometry tubes. 30 μl of Dulbecco's phosphate-buffered saline (DPBS) were added to the control tube to the cell suspension. 10 μl of Staphylococcus aureus Cowan (200 million / ml) labeled by Fluorescein5isothiocyanate and 20 μl of DPBS were mixed into the test tubes, and stirred. The tubes were incubated for 30 minutes at 37 °C, after which the cells were centrifuged at 250 g for 5 min and washed by centrifugation in 2 ml of cooled DPBS with added 0.02%EDTA. The specimens were fixed in 400 μl of DPBS–EDTA with 0.04% paraformaldehyde, theflow cytofluorimeter in the FL1 fluorescence channel was used for the assay. The results were presented as the percentage of phagocytic cells (phagocytic number) and phagocytic index (PI), which were determined by the formula:

[G-mean pos/ P-pos] – [G-mean neg/ P-neg],
where P – pos is the percentage of positive (fluorescing) cells in the experimental sample;

G-mean pos is the mean fluorescence of positive cells in the experimental sample;

P-neg is the percentage of positive cells in the negative control sample;

G-mean negisthe mean fluorescence of the negative control.

The intracellular production of reactive oxygen species (ROS) was assessed by flow cytometry [15]. The desired volume of cells was added to the flow cytometry tubes. 30 μl of DPBS was added to the suspended cells into the control tube. 4.2 μl of dichlorofluorescein (Sigma, USA) and 25.8 μl of DPBS were mixed into the test tubes, and stirred. The tubes were incubated for 30 minutes at 37 °C, and then the cells were centrifuged at 250 g for 5 min and washed by centrifugation in 2 ml of cooled DPBS with added 0.02% EDTA. The specimens were fixed in 400 μl of DPBS–EDTA with 0.04% paraformaldehyde, the assay was carried out on a flow cytofluorimeterin the FL-1 fluorescence channel. The percentage of cells producing ROS was assessed, as well as the mean fluorescence intensity (MFI) per the cell number that reflects the level of intra cellular ROS production.

Conclusion

Immune status assessment, including the phagocytic process evaluation, is a part of a comprehensive laboratory study of the patient and, as any laboratory test, is used to confirm the clinical diagnosis. In some cases, the clinical picture of the disease is so marked that on its basis it is possible to make preliminary diagnosis of the impaired phagocytic immunity. Chronic granulomatous disease can serve as an example. However, the final diagnosis can be based only on a detailed study of phagocytic process with the aid of laboratory methods.

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ЛАЗЕРНА ПРОТОЧНА ЦИТОМЕТРІЯ: ФІЗИЧНІ ОСНОВИ І ЇЇ ЗАСТОСУВАННЯ В ОЦІНЦІ ФАГОЦИТАРНОГО ПРОЦЕСУ

У даній статті розглянуті фізичні принципи методу лазерної проточної цитометрії й показані можливості даного методу для медичної й біологічної науки. Метод ефективний при вирішенні важливих завдань в оцінці фагоцитарного процесу. Вивчення показників фагоцитозу має значення в комплексному аналізі діагностики як первинних імунодефіцитних станів, так і вторинних, а саме рецидивних запальних процесів, схильності до післяопераційних ускладнень.

Ключові слова: *проточна цитометрія, нейтрофіли, фагоцитоз, реактивні форми кисню.*

М.О. Иваненко

ЛАЗЕРНАЯ ПРОТОЧНАЯ ЦИТОМЕТРИЯ: ФИЗИЧЕСКИЕ ОСНОВЫ И ЕЁ ПРИМЕНЕНИЕ В ОЦЕНКЕ ФАГОЦИТАРНОГО ПРОЦЕССА

В данной статье рассмотрены физические принципы метода лазерной проточной цитометрии и показаны возможности данного метода для медицинской и биологической науки. Метод эффективен при решении важных задач в оценке фагоцитарного процесса. Изучение показателей фагоцитоза имеет значение в комплексном анализе диагностики как первичных иммунодефицитных состояний, так и вторичных – часто рецидивирующих воспалительных процессов, склонности к послеоперационным осложнениям.

Ключевые слова: *проточная цитометрия, нейтрофилы, фагоцитоз, реактивные формы кислорода.*

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