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Fluorescent and flow cytometric analysis of cellular biochemical content of basic (cationic) cytoplasmic proteins in granulocytes

E. Zvetkova, G. Valet, E. Katzarova, E. Ianeva, A. Neronov*

Institute of Cell Biology and Morpholgy, Bulgarian Academy of Sciences, Sofia
**Max Planck Institute of Biochemistry, Munich — Germany*

Content of basic proteins in the cytoplasmic granules of granulocytes from human blood and mouse bone marrow is achieved by labelling with five sulfonated fluorescent dyes. Three of them — brilliant sulfoflavine, primuline and lucifer yellow are bound specifically to basic proteins in cytoplasmic granules of granulocytes — neutrophils and eosinophils. The staining is stoichiometric and the fluorescence intensity reflects quantitatively the content of basic cytoplasmic proteins in granulocytes. The exitation, wavelength, color of emitted fluorescence and histogrammes are characterized for each staining method. The best results are obtained with a new fluorescent dye lucifer yellow. This fluorochrome applied in our flow cytometrical studies gives basis for development of quantitative cytochemical method for flow cytometrical diagnosis of basic cytoplasmic proteins in cells of myeloid series (granulocytes from peripheral blood and their precursors from bone marrow) — in normal and pathological conditions.

Key words: basic cytoplasmic proteins, myeloid cells, granulocytes, fluorescent cytochemistry, flow cytometry, sulfonated fluorescent dyes (brilliant sulfoflavine, lucifer yellow, primuline).

Basic (cationic) proteins in the cytoplasmic granules of neutrophilic and eosinophilic granulocytes are good markers of myeloid cells from the peripheral blood and bone marrow [1, 3, 8, 13, 14]. The content of these proteins, which are also bactericidic substances, changes in cases of bacterial, viral and fungal infections, leukaemias, autoimmune diseases, cancer etc. [4-7, 9-12].

The purpose of the present study is the fluorescent and flow cytometrical analysis of basic cytoplasmic proteins in myeloid cells (neutrophils, eosinophils and their precursors) — from the human peripheral blood and mouse bone marrow, after fluorochromation with some sulfonated acid dyes. The results obtained are compared with these of our previous cytological method for staining of basic proteins by fast green, which is also acid sulfonated dye [8, 9].

Material and methods

I. Fluorescent cytochemistry

Human leukocytes from the peripheral blood smears and haemopoietic cells from mouse bone marrow smears were fluorochromated by acid sulfonated dyes (brilliant sulfoflavine, lucifer yellow, primuline, sulforodamine B or acid red 52, acid blue 93 or methylblue etc., obtained by Merck, Aldrich and Sigma). The staining was performed by different concentrations of fluorochromes — from 0,1 % to 0,001 % solutions in PBS or borate buffer (pH 7,2—8), without or after fixation of smears. After 5 min staining smears are rinsed in the same buffer, air-dried, cleared in two xylenes (30s each) and embedded in Fluormount. Fluorescentmicroscopical studies were carried out with a Zeiss universal microscope with suitable filter combinations.

II. Flow cytometry

Human leukocytes were isolated from the heparinized venous blood in suspension, rinsed in PBS and stained by the same acid sulfonated dyes. In each case 250 µl of leucocytes' suspension in PBS were mixed with 5 µl cocktail of sulfonated acid dye (1 mg/ml) and 5 µl propidium iodid (2 mg/ml) — for nuclear staining of death cells, rinsed in PBS, centrifuged and measured with FITC filter combination by techniques of flow cytometry (measurements were performed in the Max Planck Institute of Biochemistry — Munich, Germany, by Fluvo-Metricell flow cytometer).

Results and discussion

The analysis of the results from fluorescent-cytochemical studies gives the basis to conclude that three of sulfonated fluorescent dyes applied — brilliant sulfoflavine, primuline and lucifer yellow bind specifically to basic cytoplasmic proteins in myeloid cells. The chemical structures of two of these compounds are shown in Fig. 1.

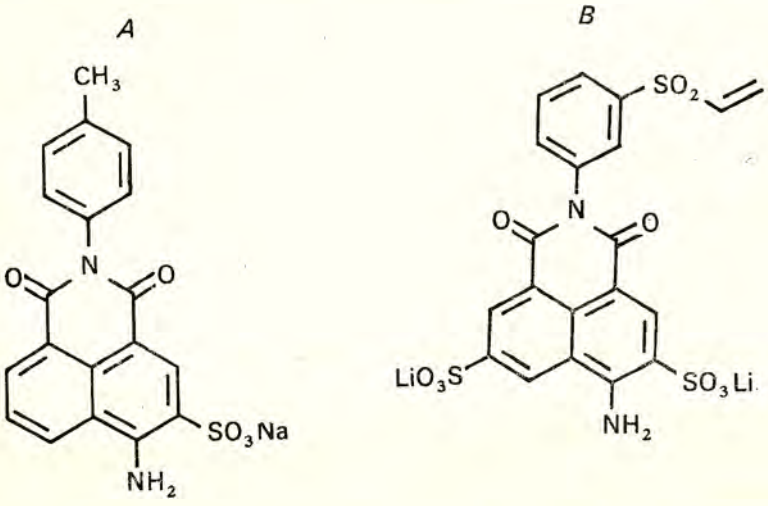

Fig. 1. The chemical structures of two sulfonated fluorochromes
A — brilliant sulfoflavine; B — lucifer yellow

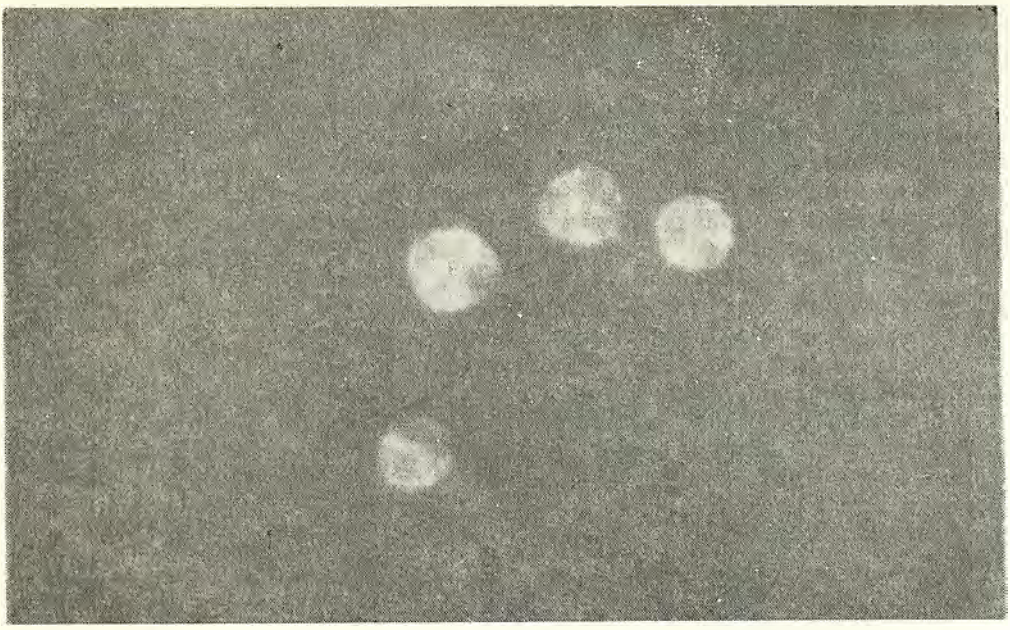

Fig. 2. Granulocytes from human peripheral blood, after fluorochromation by lucifer yellow. One can see brightly fluorescent cytoplasmic granules in neutrophils and eosinophil. Only nuclear areas are non fluorescent, × 1000. Immersion

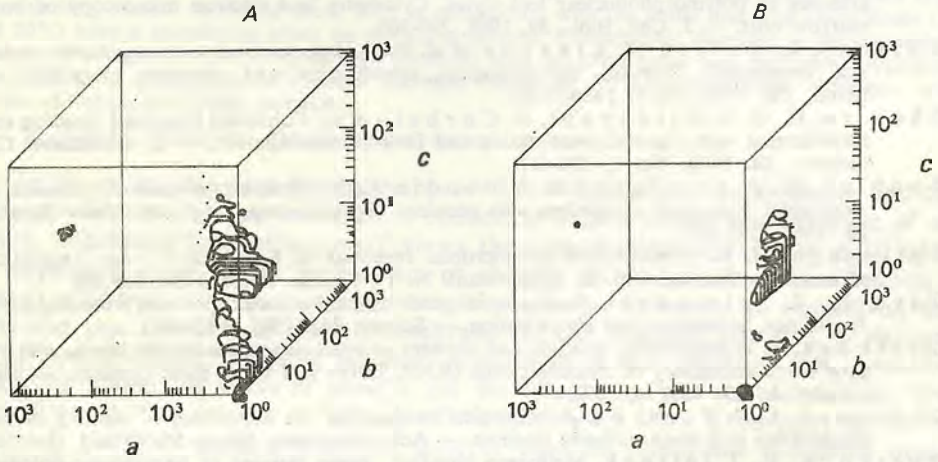

Fig. 3. Three-dimensional histogrammes of 366-610 nm fluorescence for the human leukocyte population

A — green versus red fluorescence and cell volume, after fluorochromation by lucifer yellow (LY); *B* — green versus red fluorescence and cell volume, after fluorochromation by brilliant sulfoflavine (BSF). Fluvo-Metricell flow cytometer; *a* — green fluor; *b* — volume; *c* — red fluor

The colour of fluorescence is green-yellow after fluorochromation with brilliant sulfoflavine and primuline and golden-yellow — after lucifer yellow. The fluorescent cytoplasmic granules are localised in different quantity in mature peripheral blood granulocytes — neutrophils and eosinophils (Fig. 2). The intensity of the fluorescence is more strong and fluorescent cytoplasmic granules are largest in eosinophilic cytoplasm. The staining is stoichiometric and the intensity of fluo-

rescence reflects quantitatively the content of basic cytoplasmic proteins not only in the mature blood cells from myeloid origin, but also in their bone marrow precursors.

The best cytochemical and flow cytometrical results were obtained with a new fluorescent dye lucifer yellow. The application of this fluorochrome in flow cytometrical studies (Fig. 3) gives basis for development of new quantitative method for flow cytometrical diagnosis of basic cytoplasmic proteins in leukocytes from myeloid series (granulocytes — neutrophils and eosinophils, as well as their bone marrow precursors), in normal and pathological conditions.

The results obtained are also in reasonable agreement with these of other fluorescent studies on basic proteins [2, 3, 4, 12], as well as with our previous results by the methylene blue-fast green staining method [7-11].

The conclusion is that the exitation, wavelength, color of emitted fluorescence and the histogrammes obtained with a new dye lucifer yellow, give a possible new way for cytochemical and flowcytometrical studies of basic cytoplasmic proteins in leukocytes from myeloid origin, in different states of maturation and differentiation. Cytoplasmic fluorescence values may be further analyzed to obtain the ratio of different leukocytes subtypes — e. g. neutrophils, eosinophils and their precursors.

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