

FLOW CYTOMETRIC DETERMINATION OF GRANULOCYTE FUNCTIONS
IN PATIENTS DEVELOPING SEPSIS
(PHAGOCYTOSIS, INTRACELLULAR pH AND CELL VOLUME)**

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1. INTRODUCTION

Sepsis remains a frequent complication in patients following severe trauma or major surgery. The diagnosis of sepsis is often difficult and remains a clinical problem. The purpose of this study was to develop a simple flow cytometric technique to measure different functional parameters of granulocyte phagocytosis. In addition intracellular elastase contents were measured. All data were correlated with the clinical development of the disease.

2. METHODS AND RESULTS

Buffy coat leukocytes from heparinized whole blood were incubated with K12 E. coli (100 bacteria per leukocyte) at 37°C for 0, 30 and 90 minutes.

Phagocytosis and degradation of bacterial DNA were determined by staining with acridine orange (AO) (8 ug/ml) for 15 minutes at 0°C. The DNA of dead cells was simultaneously counterstained with propidium iodide (PI) (40 ug/ml).

Intracellular pH and esterase activity (1) were measured after staining with 1,4-diacetoxy-2,3-dicyano-benzene (ADB) (25 ug/ml) for 15 minutes at 0°C and counterstaining with PI.

Elastase antigen content was visualized by a FITC-indirect-immunofluorescence assay after cell fixation in a 3.7 % (v/v) formaldehyde, 1 % (v/v) methanol, 10 mg/ml CaCl₂-solution. DNA was counterstained with PI.

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Electrical cell volume and two fluorescences of the cells in the different assays were measured simultaneously with a Fluvo-Metricell flow cytometer (HBO-100 high pressure mercury arc lamp) in list-mode operation. AO: exc. 400-500 nm, em. 500-530 nm and 550-700 nm. ADB: exc. 300-400 nm, em. 418-440 nm and 500-700 nm.

Normal Healthy Persons

The mean cell volume of the granulocytes increased by 35 to 70 % and AO green fluorescence increased by 20 to 70 % after 30 minutes of phagocytosis of *E. coli* (Fig. 1).

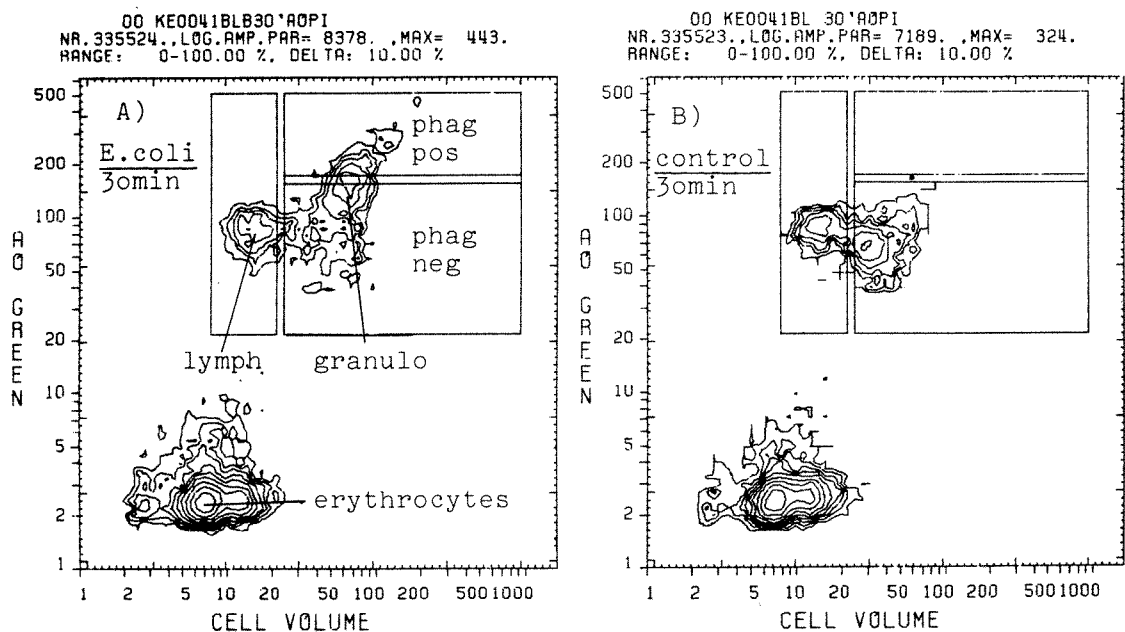


Fig. 1 Two parameter histogram (cell volume versus acridine orange green fluorescence). a) buffy coat leukocytes incubated with *E. coli* for 30 minutes, b) control

The intracellular pH was 7.5 in granulocytes and 7.6 in lymphocytes prior to incubation. It decreased by approximately 0.15 units in granulocytes but did not change in lymphocytes.

ADB-esterase activity was higher in granulocytes than in lymphocytes and decreased in granulocytes following phagocytosis.

Elastase-antibodies bound to the majority of the population of large cells consisting mainly of granulocytes (Fig. 2).

Posttraumatic and Septic Patients

Two characteristic patterns of granulocyte function different to healthy controls were observed in patients:

(a) Following severe trauma, but before onset of sepsis, granulocyte cell volume increased by 100 to 140 % and AO green fluorescence by 70 to 140 % after 30 minutes of incubation. Granulocytes internal pH fell by up to 0.6 units.

A rapid decline of granulocyte in vitro reactivity following trauma was sometimes observed and had a lead time of several days before the development of sepsis.

(b) During severe sepsis granulocytes showed reduced volume changes (+ 20 % after 30 minutes) upon incubation with bacteria. Cell volume often decreased following further incubation (+ 5 to 10 % of the initial values after 90 minutes). AO green fluorescence was decreased after 30 minutes of incubation. The intracellular pH of granulocytes decreased only moderately upon phagocytosis (pH - 0.1 units).

The percentage of elastase-positive cells in the large-volume-population of leukocytes was reduced to less than 50 % following trauma and during sepsis (75 to 95 % granulocytes in white blood cell differential count).

3. CONCLUSIONS

Flow cytometric determination of functional parameters of human granulocytes during in vitro phagocytosis of *E. coli* in 40 patients following severe trauma or during sepsis revealed three characteristic patterns of granulocyte function: a phase of early hyperactivity (increase in cell volume, AO green fluorescence and decrease of intracellular pH), a phase of normal reactivity and a phase of granulocyte hypo- or anergy in severe sepsis.

The flow cytometric parameters were in a significant number of patients more sensitive for the assessment of the development of the disease than standard parameters determined by the clinical laboratory such as total leukocyte count, differential white blood cell count etc..

The onset of changes of functional granulocyte parameters often occurred several days earlier than the deterioration of the patients' condition. The functional granulocyte abnormalities were suitable for automated detection by the DIAGNOS1 program system.

1. Valet, G., Raffael, A., Moroder, L., Wuensch, E., Ruhenstroth-Bauer, G. (1981). *Naturwissenschaften* 68, 265-266.

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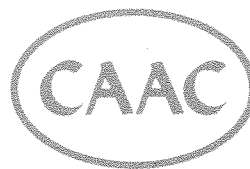
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