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Flow cytometric study on phagocyte function and determination of in vitro natural killer (NK) cell cytotoxicity in hairy cell leukaemia (HCL)

Comparison with values obtained following post-traumatic splenectomy (SP)

JUDIT DEMETER, R MIHALIK, KATALIN PÁLÓCZI, M BENCZUR

National Institute of Haematology and Blood Transfusion, Budapest

The cause of the infection susceptibility of HCL as well as splenectomized patients was studied.

Patients. Twelwe untreated patients were studied, two females with HCL treated formerly by splenic radiotherapy and SP, respectively, as well as one female and 9 male patients 2 to 17 years following posttraumatic SP. Eleven healthy age and sex-matched volunteers served as controls.

Methods. 1. Phagocytosis and flow cytofluorometry (FCM). The method of Hed et al. [1] was used with some modifications. FITC-labelled yeast particles preopsonized with normal human serum (designated FITC-yeast-C3b) were incubated for two hours at 37 °C with the leukocyte pellet obtained from EDTA blood by lysis of RBCs with NH_4Cl "Lysing Reagent" (Ortho) and centrifugation. The concentration of granulocytes as well as of FITC-yeast-C3b particles was 5×10^6/ml. Analysis was done in a FACSTAR flow cytometer (Beckton-Dickinson). Frames were set around the granulocyte and the mononuclear leukocyte cell cluster. Trypan blue fluorescence quenching was used to differentiate between attached and ingested particles (Fig. 1). 2. NK cytotoxicity was determined by measuring Cr51 release from K562 target cells.

RESULTS AND DISCUSSION

Data are shown in Table I. HCL: The hairy cells (HCs) did not phagocytose FITC-yeast C3b, proved also on purified (E + depleted), though Mo-2+ HCs from patient 1. NK cytotoxicity was reduced corresponding to clinical stage [2]. Posttraumatic SP: Phagocytosis of yeast-C3b particles and NK cytotoxicity were found to be normal. The role of an opsonisation defect is investigated. Preliminary data show that FCM of phagocytosis from unseparated peripheral blood is a simple and informative technique for

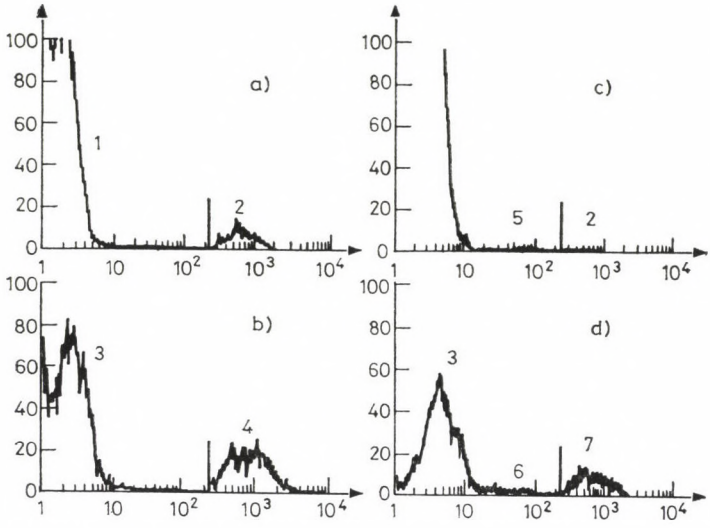

FIG. 1. Fluorescence histograms, *A:* Free FITC-conjugated yeast-C3b particles. *B:* Granulocytes interacting with FITC-yeast-C3b particles (attached and/or ingested). The quenching effect of trypan blue on FITC-yeast-C3b particles *(C)* and on particles interacting with phagocytosing granulocytes *(D)* 1: red blood cell lysate 2: free FITC-yeast-C3b particles 3: non-phagocytosing granulocytes 4: granulocytes interacting with yeast-C3b (attached and/or ingested) 5: quenching of yeast-C3b fluorescence 6: quenching of FITC-yeast-C3b particles attached to granulocytes 7: granulocytes phagocytosing FITC-yeast-C3b

TABLE I

Percentage of phagocytosing granulocytes, mononuclear leukocytes and percent specific release from K562 cells at an E : T ratio of 25 : 1 (calculated from the cytotoxicity curve)

	Granulocytes	Mononuclears	NK cytotoxicity
Controls (n = 11) ($\bar{x} \pm$ SD)	24.2±4.7	0.6±0.28	45.8±11.4
Posttraumatic splenectomy (n = 10)	23.3±5.4	0.3±0.25	51.8±17.3
HCL			
patient 1	17.5	0.2	11.3
patient 2	3.8	0	6.7

screening of phagocytosis defects. In HCL it makes separation of HCs unnecessary.

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