GENERAL PATHOLOGY AND PATHOLOGICAL PHYSIOLOGY

Expression of Bone Marrow Macrophage Receptors in Cytostatic-Induced Myelodepression

E. D. Goldberg*, Yu. P. Bel'skii, M. G. Danilets, A. M. Dygai*, V. V. Zhdanov*, and S. A. Kusmartsev

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Expression of macrophage sialoadhesin and erythroblast receptors involved into the hemopoietic islet formation is studied in cyclophosphamide-treated CBA mice. The number of hemopoietic islets and the content of immature granulocytic and erythroid cells were determined. Cyclophosphane reduces the expression of sialoadhesin and erythroblast receptors. It can be hypothesized that the disturbances in granulocyte differentiation result from impaired sialoadhesin-mediated interaction between hemopoietic cells and macrophages.

Key Words: *sialoadhesin; erythroblast receptor; macrophage; hemopoietic islets; cyclophosphamide*

It has been previously shown that alkylating antitumor agents induce considerable structural and functional changes in hemopoietic tissues [2]. Stromal cells (macrophages, fibroblasts, endotheliocytes, etc.) play an important role in the regulation of normal and disturbed hemopoiesis via selective cell-cell interactions mediated by various adhesion molecules. For instance, macrophages possess specific surface receptors: sialoadhesin and erythroblast receptor (ER) [6,12]. Sialoadhesin (185 kD) is a macrophage-restricted receptor, a member of the immunoglobulin superfamily, structurally similar to CD22 [9]. This receptor recognizes and binds specific sialylated ligands on bone marrow granulocytic cells [3,7] and some lymphocyte subpopulations, in particular, splenic lymphocytes [14]. Erythroblast receptors are less studied. This receptor mediates the interaction between macrophages and immature erythroid cells, but not between differentiated macrophages and erythrocytes. It has been shown that the adhesion molecule VCAM-1 expressed on macrophages plays an essential role in hemopoietic islet (HI) organization through the binding to VLA-4 integrin on erythroblasts [13].

The aim of the present study was to evaluate the role of sialoadhesin and ER expressed on macrophages in the regeneration of erythro- and granulocytopoiesis in mice injected with cyclophosphamide (CP).

MATERIALS AND METHODS

Experiments were carried out on 110 CBA/CaLac mice aging 2-2.5 months (grade I, conventional strain, collection of the Laboratory of Experimental Biomedical Modeling, Tomsk Research Center).

Cyclophosphamide (Biokhimik, Saransk) was injected intraperitoneally in a dose of 250 mg/kg. Control animals received an equivalent volume of physiological saline. Expression of macrophage receptors was evaluated using the rosette-formation test [5]. Myelokaryocytes were isolated from the

Laboratory of Experimental Biomedical Modeling and *Institute of Pharmacology, Tomsk Research Center, Siberian Division of the Russian Academy of Medical Sciences

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TABLE 1. Expression of Sialoadhesin and ER on Bone Marrow Macrophages from CP Treated Mice ($X \pm m$)

Days after injection	Macrophages, %			
	sialoadhesin-bearing		ER-bearing	
	control	experiment	control	experiment
1	80.5±11.5	57.5±2.5	81.0±4.3	71.0±2.0
3	82.33±4.0	67.5±5.3	54.0±1.1	29.5±1.5**
5	84.0±4.7	49.5±5.3*	74.5±2.2	63.5±1.8
7	83.8±6.8	75.8±4.0	81.0±2.7	68.3±1.5

Note. *$p<0.002$, **$p<0.001$ compared with the control.

femur with phosphate buffered saline containing 0.05% collagenase and washed 3 times. The cells were resuspended in RPMI-1640 medium containing 10% fetal serum, transferred to slides, and incubated at 37°C and 5% CO_2 for 3 h. Nonadhesive cells were removed, and sialoadhesin- and ER-specific ligands (sheep erythrocytes and erythroid cells from 13-15-day-old mouse embryos, respectively) were added to the monolayer. The preparations were fixed with glutaraldehyde and stained with azure II-eosin; rosettes were counted under a light microscope.

To analyze the structure and function of the bone marrow, HI were enzymatically isolated, stained with Neutral Red, and counted in a Goryaev chamber. This dye is absorbed primarily by macrophages and hence visualizes macrophage-containing HI. Myelogram was read on smears stained with azure II-eosin.

The data were processed statistically using the Student t test.

RESULTS

Injection of CP suppressed the expression of macrophage receptors: the percentage of sialoadhesin-bearing cells decreased on day 5 and returned to the baseline on day 7 after injection, while the decrease and recovery of ER-bearing macrophages occurred on days 3 and 5, respectively (Table 1). This reduced the hemopoiesis-modulating capacity of bone marrow macrophages, since these receptors mediate the binding of hemopoietic elements in HI, where they proliferate and differentiate from committed precursors to mature cells [4].

The content of HI in the bone marrow decreased on day 3 and returned to normal level on day 5 after injection (Table 2). The content of immature neutrophil granulocytes decreased immediately after injection (Table 2); on day 3 it constituted 15-17% of normal value and then sharply rose, considerably surpassing the control level. The sharp rise of immature neutrophils coincided with reduced sialoadhesin expression.

Erythroblasts disappeared from the bone marrow immediately after injection and, unlike granulocytes, the number of these cells was negligible throughout the experiment (Table 2).

It is known that normal hemopoiesis is regulated through the interaction between hemopoietic cells and bone marrow stromal elements. Decreaseded expression of sialoadhesin on day 5 after injection and a simultaneous rise of immature granulocytes together with normal content of HI suggest an imbalance between granulocyte proliferation and differentiation due to weakened macrophage influence on hemopoietic cells bound to macrophage though sialoadhesin. Our assumption is consistent with the data on the involvement of sialoadhesin into the granulocyte differentiation [10,11].

TABLE 2. Number of Immature Neutrophils, Erythrokaryocytes, and HI in the Bone Marrow of CP-Treated Mice (% of Control, $X \pm m$)

Days after injection	Immature neutrophils	Erythroid cells	Number of HI per femur, $\times 10^3$
Before injection	100.00±4.81	100.00±8.42	100.00±26.88
1	15.70±1.48**	1.95±0.18**	51.75±17.41
3	17.70±2.22**	0.37±0.18**	22.58±3.76*
5	280.93±18.15**	8.76±1.58**	101.61±18.82
7	259.48±30.37**	9.37±2.11**	77.15±13.17

Note. *$p<0.05$, **$p<0.001$ compared with initial values.

The number of erythroid cells was stably suppressed at all terms after CP injection against the background of ER and HI variations. We assumed that CP inhibited the early stages of erythropoiesis and blocked the formation of erythroblasts which can bind to macrophage through ER. These findings on ER-mediated interaction are essential for terminal stages of erythropoiesis before the release of enucleated cells into circulation [8]. The effect of CP on the extracellular matrix of the bone marrow playing an important role in erythropoiesis cannot be excluded.

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