Early Blood System Response to Infectious Agents

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Early systemic blood responses to various infectious agents (*Staphylococcus aureus, Escherichia coli,* herpes simplex virus) were studied. Nonspecific changes in the blood typical of stress syndrome and enhanced apoptosis of in the bone marrow and lymphoid organs were seen 6 hours after injection of bacterial agents. Injection of *E. coli* induced marked depression of hemopoietic precursors and extensive apoptosis of bone marrow, thymic, and splenic cells. Herpes simplex virus induced only minor changes in the blood system.

Key Words: *hemopoiesis; Staphylococcus aureus; Escherichia coli; herpes simplex virus*

Infectious process is often accompanied by pronounced changes in the hemopoietic system [2,3,7,8] due to direct cytotoxic effect of microbial toxins on blood cells [7,8] and structural and functional changes in hemopoiesis-inducing microenvironment [2,3]. Moreover, injection of infectious agents is associated with stress and accompanied by peculiar changes in the blood [2]. The effect of most stress factors are realized though induction of apoptosis [1,4,6]. This process takes several hours (from chromatin aggregation to complete degradation of apoptosis bodies) [6]. Therefore it was interesting to study early hemopoietic responses to various infectious agents and the role of apoptosis in their realization.

The aim of the present study was to evaluate early responses of the blood system to various infectious agents.

MATERIALS AND METHODS

Experiments were carried out on 2-month-old CBA/CaLac mice weighing 18-20 g (n=40, Rassvet breeding center, Tomsk). Experimental animals were intraperitoneally injected with a suspension of infectious agents (in 0.5 ml physiological saline): *Staphylococcus aureus* (strain B 243) in a dose of $^1/_2$ LD_{50} (2×10^8 microbial bodies), *Escherichia coli* (strain N 304) in a dose of $^1/_2$ LD_{50} (4×10^8 microbial bodies) and in maximal permissible dose (MPD=4×10^7) and herpes simplex virus (L2) in a dose of LD_{50} (virus dilution 10^{-4}). Control mice received 0.5 ml intraperitoneal saline. Parameters of peripheral blood (erythrocytes, 10^{12}/liter; reticulocytes, %; leukocytes, 10^9/liter) were analyzed 6 hours postinjection. After sacrifice by cervical dislocation, total cell count in the bone marrow and myelogram (10^6/femur) were analyzed, as well as the thymus and spleen were weighted. On azur II-Nocht-stained cytological preparation of the bone marrow, thymus and spleen, the percentage of apoptotic bodies and

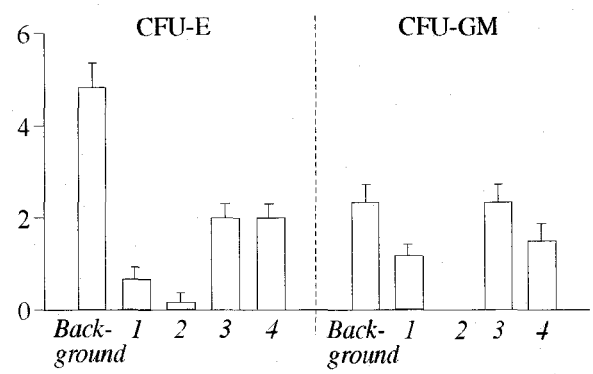

Fig. 1. Dynamics of precursors of erhythro-(CFU-E) and granulomonocytopoiesis (CFU-GM) in CBA mice 6 h after infection. Here and on Fig. 2: *Background*: 0.9% NaCl, 0.5 ml; *1*: *S. aureus*, $^1/_2$ LD_{50}; *2*: *E. coli*, $^1/_2$ LD_{50}; *3*: *E. coli*, maximal permissible dose; *4*: herpes simplex virus, $^1/_2$ LD_{50}. Ordinate: Number of CFU-E and CFU-GM per 10^5 myelokaryocytes.

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TABLE 1. Parameters of Peripheral Blood in CBA Mice 6 h after Infection ($X \pm m$)

Agent	Erythrocytes, 10^{12}/liter	Reticulocytes, %	Total number	Neutrophils	Lymphocytes
			10^9/liter		
0.9% NaCl, 0.5 ml	8.67±0.49	54.3±2.0	8.23±1.33	3.76±0.58	4.05±0.82
S. aureus, $^1/_2$ LD_{50}	7.66±0.23	79.7±8.1*	14.03±0.52*	8.64±0.15**	4.48±0.59
E. coli, $^1/_2$ LD_{50}	8.85±0.50	44.3±4.9	14.07±1.38*	3.69±0.28	9.13±1.10*
E. coli, MPD	8.56±0.42	53.7±13.2	7.61±0.72	3.81±0.51	2.38±0.69*
HSV, $^1/_2$ LD_{50}	7.51±0.12	72.0±2.9**	11.53±1.58	4.87±0.76	6.06±0.71

Note. Here and in Table 2: *$p<0.05$, **$p<0.01$ compared with NaCl (Student t test [5]).

TABLE 2. Parameters of Bone Marrow (10^6/Femur) in CBA Mice 6 h after Infection ($X \pm m$)

Agent	Total number of myelokaryocytes	Neutrophils		Lymphocytes	Erhythronormoblasts
		mature	immature		
0.9% NaCl, 0.5 ml	12.1±1.01	0.93±0.09	3.49±0.56	2.09±0.04	3.34±0.35
S. aureus, $^1/_2$ LD_{50}	10.0±1.9	0.81±0.24	2.29±0.57	2.16±0.49	3.35±0.84
E. coli, $^1/_2$ LD_{50}	10.13±0.58	0.79±0.07	1.48±0.25**	2.71±0.37	3.41±1.73
E. coli, MPD	9.50±1.04	0.65±0.09	1.40±0.27**	2.76±0.25	3.11±0.33
HSV, $^1/_2$ LD_{50}	13.53±0.32	0.98±0.01	3.92±0.24	3.34±0.55*	3.23±0.54

cells (pyknosis, fragmentation, margination of the nucleus, packing of apoptotic bodies) were assessed as described elsewhere [9]. The content of committed precursors of erhythro- and granulomonocytopoiesis (CFU-E and CFU-GM) was *in vitro* evaluated in methyl cellulose culture of bone marrow nuclears as described previously [10].

RESULTS

Injection of *Staphylococcus aureus* and herpes simplex virus (HSV) caused reticulocytosis 6 h postinjection, the number of erythrocytes being unaffected. Infection had no effect on the content of erythronormoblasts in the bone marrow, but sharply suppressed the formation of CFU-E in culture, which was most pronounced after injection of *S. aureus* and *E. coli* in a dose of $^1/_2$ LD_{50} (Fig. 1).

Changes in myelopoiesis were less pronounced. Bacterial agents ($^1/_2$ LD_{50}) caused pronounced leukocytosis (Table 1) due to the increase in neutrophil (staphylococcal infection) or lymphocyte count (enteral infection). In the bone marrow, the content of immature neutrophils decrease during bacterial infection and lymphocytosis was found during viral infection (Table 2). All agents in a sublethal dose suppressed the formation of CFU-GM in culture. Injection of $^1/_2$ LD_{50} *E. coli* completely eliminated granulocytic precursors, while this agent injected in a dose corresponding to MPD had no effect on this parameter (Fig. 1).

The weight of the thymus in animals infected with *S. aureus* and *E. coli* significantly decreased in comparison with the control (to 29.0±1.0 and 25.7±2.1 mg, respectively, *vs.* 33.6±1.1 mg in the control), but did not differ significantly from that in mice injected with saline (23.3±3.6 mg). The weight of the spleen was similar in all animal groups (78.7±16.8 mg).

Morphological analysis showed accumulation of apoptotic cells and apoptotic bodies in the bone marrow and lymphoid organs during infection (Fig. 2). Injection of *E. coli* caused most pronounced increase in the content of apoptotic cells in the thymus, bone marrow, and spleen. *S. aureus* stimulated the formation of apoptotic bodies in the thymus and spleen. HSV induced no apoptosis in the examined organs 6 h postinjection.

Thus, 6 h after injection of infectious agents we observed nonspecific changes in the blood system characteristic of stress syndrome, such as enhanced apoptosis, most pronounced in the thymus. The hallmark of early hemopoietic response to infection was a pronounced decrease in the content of bone marrow precursors. Considerable dose-dependent inhibition of hemopoietic cells and enhanced apoptosis induced by *E. coli* are most probably a specific effect of this microorganism.

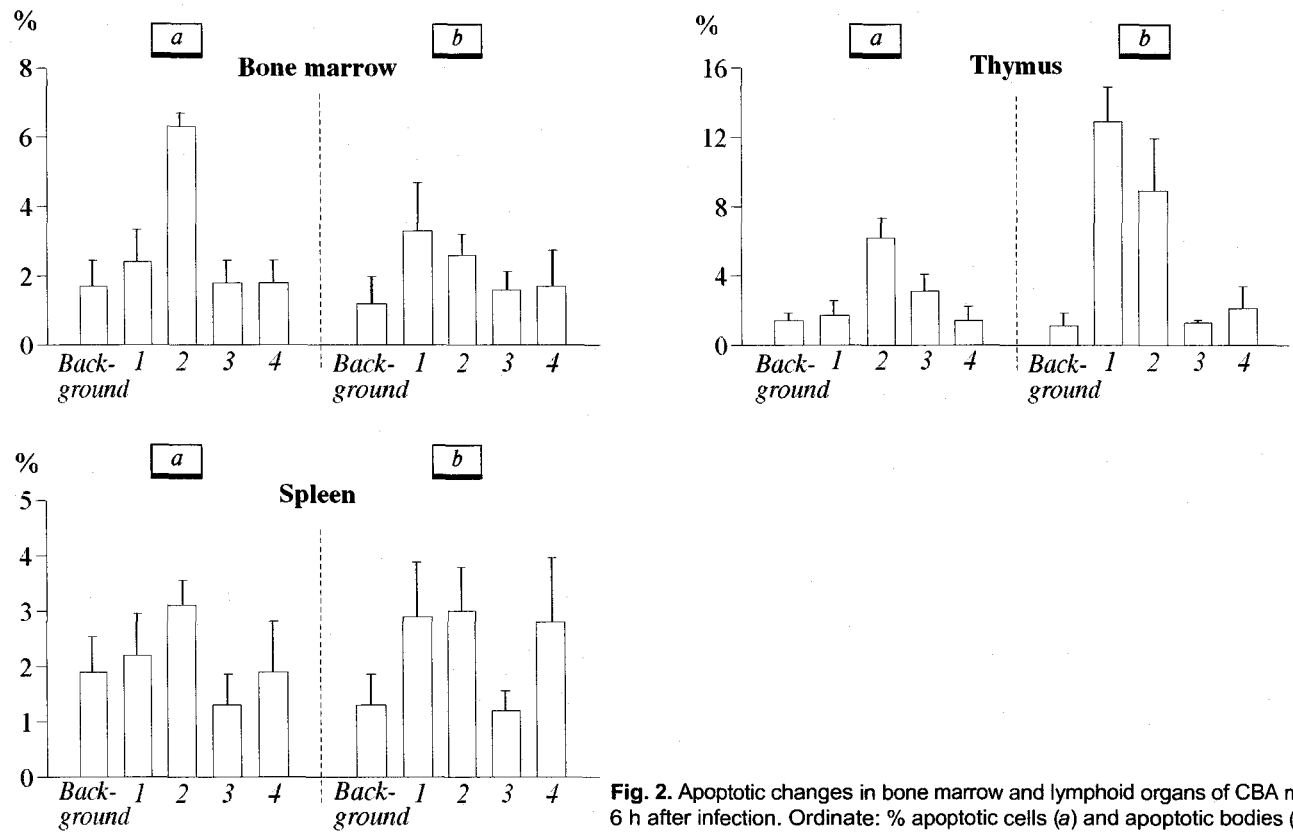

Fig. 2. Apoptotic changes in bone marrow and lymphoid organs of CBA mice 6 h after infection. Ordinate: % apoptotic cells (*a*) and apoptotic bodies (*b*).

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