Erythropoiesis in Bacterial Infection

M. R. Karpova, V. V. Zhdanov, O. I. Urazova, T. V. Kashtanova, and M. V. Shamis

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The reaction of erythroid hemopoietic stem to bacterial agents (*E. coli* and *St. aureus*) is studied. Suppression of bone marrow erythropoiesis in infected mice is caused by disorders in mitotic activity of erythroid elements.

Key Words: *erythropoiesis; bacterial infection; Escherichia coli; Staphylococcus aureus*

The reactions of blood system to infectious agents have been investigated in sufficient detail [5,6,8,12]. There are numerous reports on stimulation of the myeloid hemopoiesis stem [2,6,8]. However, the effects of pathogenic microorganisms on erythropoiesis are still not quite clear. There is evidence that the erythroid hemopoietic stem is suppressed [4,5] or activated [2,7] by infectious agents.

We studied the reaction of the erythroid stem to an infectious process caused by different microorganisms.

MATERIALS AND METHODS

Experiments were carried out on 200 outbred male albino mice aged 8-12 weeks from Rassvet Breeding Center, Tomsk. *St. aureus* B243 and *E. coli* H304 strains were used. *St. aureus* (strain B243) possesses plasmacoagulase, DNAse, hyaluronidase, fibrinolysin, flocculating factor, produces α- and δ-toxins, is virulent for mice, and belongs to the third phage group (phage type 83 A). It was obtained from the Department of Staphylococcal Infections, N. F. Gamaleya Institute of Epidemiology and Microbiology, Russian Academy of Medical Sciences. *E. coli* (strain 034 K-H10 N H304) was isolated by Knipschildt in 1945 from normal human feces. This strain is characterized by typical escherichia enzymatic properties and is not antigenically related to *Shigella, Salmonella,* and other enterobacteria. The strain was obtained from the Collection of the Department of Microbiology of Opportunistic Bacteria, I. I. Metchnikoff Institute of Vaccines and Sera, Russian Academy of Medical Sciences. For inducing an infectious process, a suspension of microorganisms in normal saline was injected intraperitoneally in a dose of $^1/_2$ LD_{50}, which was 10^9 live bacteria for *St. aureus* and 2×10^6 for *E. coli.* After one week the mortality of mice infected with *St. aureus* B 243 was 22.1% and with *E. coli* 25.3%. On days 2, 3, 5, 7, 10, 14, 21, and 28 after infection the counts of peripheral blood erythrocytes and reticulocytes, total counts of karyocytes, and myelogram were determined on bone marrow preparations stained according to Nocht-Maximov [4]. The count of erythropoietic committed precursor cells (CFU-E) was determined by cloning bone marrow nuclear cells in methyl cellulose as described elsewhere [10]. Erythropoietic activity (EPA) of adherent and nonadherent bone-marrow cells was studied *in vitro* on intact mouse myelokaryocytes [1]. Splenic erythropoietic was assessed on splenograms (on spleen preparations stained according to Nocht-Maximov). Proliferative activity of bone-marrow cells was assessed by counting statmokinetic indices [3]. For evaluating the agent inoculation rate, liver and spleen were removed under sterile conditions, homogenized, the suspension was diluted 1000-fold with normal saline, and transferred on Petri dishes with agar. Blood collected from the heart under sterile con-

Siberian State Medical University, Tomsk; Institute of Pharmacology, Tomsk Research Center, Siberian Division of the Russian Academy of Medical Sciences

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TABLE 1. Erythropoiesis Values in Mice Infected with *St. aureus* in $^1/_2$ LD_{50} ($X \pm m$)

Day of analysis	Reticulocytes, ‰	Total karyocyte count, 10^6/femur	Erythrocytes, karyocytes, 10^6/femur	Statmokinetic index of erythrokaryocytes	CFU-E, per 10^5 myelokaryocytes	Adherent cell EPA	Nonadherent cell EPA	Erythroid splenocytes, %
						number of colonies per 10^5 nonadhesive intact mouse myelokaryocytes		
Preinfection	52.00±3.11	21.10±0.60	4.76±0.86	0.11±0.05	2.70±0.30	0.31±0.12	0.19±0.10	10.10±2.10
2	50.17±3.23	17.33±0.97**	3.27±1.45	0.02±0.01*	8.00±0.90**	1.00±0.38	1.38±0.38*	1.30±0.70*
3	43.67±4.36	26.46±1.57**	3.92±1.01	0.03±0.03	5.50±1.10*	0.50±0.27	1.25±0.25**	6.60±2.30
5	55.20±4.45	17.25±1.29*	1.64±0.91*	0.05±0.03	2.80±0.40	0.75±0.25	0.38±0.18	27.20±5.90*
7	60.00±5.91	20.58±1.23	2.41±1.05*	0.03±0.03	4.00±0.80	0.63±0.26	1.50±0.33**	25.20±5.00*
10	60.00±6.90	14.75±1.33**	1.09±0.05*	0.03±0.03	0.80±0.40**	1.25±0.37*	0.67±0.21	14.00±3.10
14	56.25±1.22	30.50±2.88**	5.97±2.11	0.03±0.03	3.60±0.70	0.50±0.19	0.63±0.26	6.00±0.80
21	49.00±0.77	17.13±1.90	2.19±0.86*	0.03±0.04	1.70±0.30	0.50±0.38	0.38±0.18	11.20±2.50
28	45.17±1.76	22.90±2.21	4.76±1.59	0.03±0.05	0.70±0.30	0.25±0.16	0.71±0.28	14.10±2.80

Note. Here and in Table 2: *$p<0.01$ *vs.* preinfection values.

TABLE 2. Erythropoiesis Values in Mice Infected with *E. coli* in $^1/_2$ LD_{50} ($X \pm m$)

Day of analysis	Reticulocytes, ‰	Total karyocyte count, 10^6/femur	Erythrocytes, karyocytes, 10^6/femur	Statmokinetic index of erythrokaryocytes	CFU-E, per 10^5 myelokaryocytes	Adherent cell EPA	Nonadherent cell EPA	Erythroid splenocytes, %
						number of colonies per 10^5 nonadhesive intact mouse myelokaryocytes		
Preinfection	52.00±3.11	21.11±0.60	4.76±0.81	0.11±0.05	2.70±0.30	0.31±0.12	0.19±0.10	10.10±2.10
2	40.60±2.25*	14.42±1.26**	1.09±0.13**	0.05±0.01*	8.60±0.70**	0.40±0.21	0.50±0.19	8.70±6.80
3	43.33±1.23*	11.80±1.60**	1.64±0.42**	0.05±0.01*	8.80±1.40**	0.71±0.29	0.38±0.18	24.00±3.30*
5	53.50±3.16	20.54±1.98	2.23±0.31*	0.05±0.01	4.20±0.50*	1.13±0.23*	1.00±0.27*	23.60±2.90*
7	63.00±1.38**	20.50±1.65	2.44±0.39*	0.03±0.01	4.80±0.80*	0.38±0.18	0.50±0.27	22.40±7.00
10	51.20±1.85	23.11±1.54	4.56±0.43	0.06±0.01	1.20±0.30**	2.63±0.32**	1.00±0.37	4.10±1.10*
14	47.80±1.66	22.40±1.92	5.36±0.58	0.05±0.01	2.20±0.60	0.75±0.25	1.00±0.27*	19.50±3.70*
21	43.17±0.79*	23.17±3.07	4.35±0.49	0.05±0.01	2.50±0.20	0.38±0.38	0.25±0.16	13.00±3.50
28	46.00±0.93	23.21±1.54	4.39±0.42	0.04±0.01	2.20±0.50	0.25±0.16	0.80±0.58	20.50±4.40*

ditions was added to 1% sugar broth. Insemination of organs and blood was considered positive if colonies were grown in agar and the broth became opaque. The agents were identified by morphological and culturing properties. The data were processed using variation statistics methods and Student's *t* test.

RESULTS

A single intraperitoneal injection of one-day culture of *St. aureus* and *E. coli* in a dose of $^1/_2$ LD_{50} led to the development of infection. Its manifestation coincided in time with the inoculation period of infectious agents from the liver, spleen, and blood of infected animals. *St. aureus* was isolated from peripheral blood and liver on days 2-7 and from the spleen on days 3-7 of experiment. *E. coli* was detected in organs and blood of infected mice starting from day 2 postinoculation. The agent persisted up to day 7 in the liver and up to day 3 in the spleen and blood.

Infection of mice with *St. aureus* caused no significant changes in peripheral blood erythrocytes and reticulocytes. The total count of karyocytes in the bone marrow decreased because of a drop in the count of erythroid cells on days 5-21 (by 50% on average, in comparison with the initial values, Table 1). At the same period degenerative erythroid cells appeared in the bone marrow (pyknosis and nuclear vacuolation, cytoplasmic vacuolation and lysis). Bone-marrow erythropoietic hypoplasia was associated with a decrease in the statmokinetic index of erythroid cells throughout the entire experiment. The content of CFU-E in hemopoietic tissue increased at early terms of experiment, which might be due to stress caused by inoculation of live microorganisms in a high dose (10^9 microbial bodies). On day 10 this value decreased. EPA production by adherent and nonadherent bone-marrow cells was enhanced mainly during the first week of experiment. After a short depression, the erythropoietic function of the spleen was activated, as evidenced by a 2.5-fold increase in the erythronormoblast count on days 5-7 of experiment (Table 1).

Infection of mice with *E. coli* did not change the erythrocyte count in peripheral blood. The count of reticulocytes decreased on days 2 and 3, normalized by day 5, and on day 7 was statistically higher than before infection (Table 2). The count of erythrokaryocytes in the bone marrow of mice infected with *E. coli* was significantly lower during the first week of experiment; degenerative cells appeared in smears. The statmokinetic index of erythroid cells was decreased during the entire observation period. The content of CFU-E increased at the beginning of experiment and decreased on day 10, after which it normalized. EPA production by adherent and nonadherent bone marrow cells increased on days 5-10 and 5-14 of experiment, respectively. The count of erythroid cells in the spleen was increased over the entire observation period (Table 2).

Thus, depression of bone marrow erythropoiesis in infected mice is caused by disorders in mitotic activity of erythroid elements. These changes may result from direct cytolytic effect of bacterial toxins toward these bone marrow cells. This hypothesis is confirmed by the presence of degenerative hemopoietic cells and by coincidence of the terms of the decrease in the count of erythrokaryocytes and isolation of microorganisms from the organs. The damage inflicted by bacteria to hemopoietic elements depends on their pathogenicity. *St. aureus* is more pathogenic than *E. coli* and causes more severe injury to hemopoietic cells. Increased EPA production by the microenvironment elements is a compensatory reaction of the bone marrow to suppression of the erythroid stem. The lack of changes in erythrocyte count in peripheral blood in response to depression of erythrokaryocyte count may be due to increased erythropoietic function of the spleen, which is an active hemopoietic organ in rodents [11] responsible for hemopoiesis in many diseases [9].

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