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PROLIFERATION OF HEMATOPOIETIC STEM CELLS AND THEIR MIGRATION INTO THE MOUSE SPLEEN AFTER A SINGLE INJECTION OF *Mycoplasma arthritidis*

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UDC 616.98:579.88]-092.9-07:616.411-018.1-097

KEY WORDS: *Mycoplasma arthritidis*, hematopoietic stem cells, S phage, f fraction.

The continuing spread of mycoplasmas and broadening of the spectrum of pathology for which they are responsible has increased interest in these microorganisms. Contaminating mycoplasmas, as intracellular and membrane parasites, can not only induce pathological cellular reactions, but can also have an effect on the results of experimental research. Despite induction of cellular or humoral immune protective responses, many mycoplasmas are capable of long persistence in the tissues of the carrier organism, and in particular, in the hematopoietic and lymphoid tissues, as has been shown for *Mycoplasma arthritidis* [4].

The aim of this investigation was to study the reaction of the hematopoietic tissue of mice and their hematopoietic stem cells (CFU) in response to a single injection of living *M. arthritidis*. The results of the initial experiments on this problem were published previously [2, 5].

EXPERIMENTAL METHOD

Male CBA/Ca ($H\text{-}2^k$) and BALB/c ($H\text{-}2^d$) mice aged 8-12 weeks, obtained from the "Stolbovaya" Nursery, Academy of Medical Sciences of the USSR, were used in the experiments.

Strain PG6 of *M. arthritidis* was obtained and kept as described previously [2]. The mycoplasmas were injected intraperitoneally into the mice in a dose of 10^8 CFU/mouse. Growth medium for mycoplasmas was injected into the control mice.

To determine the number of CFU-s, hydroxyurea was injected intraperitoneally into the mice in the S phase of the cell cycle in a dose of 500 mg/kg body weight. After 1 h the mice were killed by cervical dislocation, the bone marrow was removed from the femora, and the number of CFU-s was determined by exocolonization of the spleen of lethally irradiated syngeneic recipients [14]. The number of CFU-s in the phase of DNA synthesis was determined by the formula:

$$A = \frac{a-b}{a} \times 100$$

where A is the number of CFU-s in the S phase (in percent), a, the number of colonies formed by CFU-s of intact mice, and b the number of colonies formed by CFU-s of mice receiving hydroxyurea.

N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR P. A. Vershilova.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 102, No. 7, pp. 89-92, July, 1986. Original article submitted April 19, 1985.

TABLE 1. Number of CFU-s of Bone Marrow of CBA Mice in S Phase of Cell Cycle 24 h after Infection with *M. arthritidis*, Depending on Dose of Mycoplasmas

Dose of M. arthritidis, CFU/ml	No. of expt.	Number of colonies, M ± m		Number of CFU-s S phase, per cent
		without hydroxyurea	with hydroxyurea	
—	1	13,6±0,8 (10)	13,0±0,9 (8)	4,4
	2	10,8±1,2 (10)	11,1±1,3 (8)	0
10^5		13,6±2,8 (5)	12,9±1,2 (8)	5,1
10^6	1	13,1±1,5 (8)	12,8±1,3 (8)	2,3
	2	15,1±1,9 (9)	14,3±1,2 (6)	5,1
10^7	1	14,4±0,8 (10)	10,8±1,6 (8)	25,0
	2	19,5±1,5 (10)	11,4±2,1 (8)	41,7
10^8	1	14,0±1,2 (8)	9,4±1,2 (10)	32,9
	2	13,3±1,0 (9)	6;2±1,4 (6)	53,4

Legend. Here and in Table 2, number of mice given in parentheses.

TABLE 2. Effect of Infection of Mice Donating Bone Marrow with *M. arthritidis* on Seeding Efficiency (f fraction) of CFU-s Determined 24 h after Infection

Treatment of donors	No. of expt.	Number of CFU-s in one-third of a spleen (R_3)	Number of CFU-s in 5×10^4 bone marrow cells (R_1)	Value of f
—	1	27,5±1,9 (8)	13,1±1,1 (10)	15,7
	2	21,5±1,8 (11)	11,5±1,2 (8)	14,0
Injection of M. arthritidis	1	13,2±1,2 (11)	14,3±1,1 (9)	6,9
	2	15,4±2,4 (9)	20,1±2,8 (8)	5,7

In the experiments with endogenous colony formation the mice were irradiated in a sublethal dose (5.5-6.0 Gy). On the 9th day after irradiation the mice were killed and the spleen fixed in Bouin's solution. Macroscopically visible colonies were counted with the aid of a magnifying glass.

The f fraction of bone-marrow CFU-s migrating into the spleen was determined by the clasical method [13]. The recipients of group 1 (R_1), lethally irradiated beforehand, were given an intravenous injection of $5\cdot10^4$ syngeneic bone marrow cells. The recipients of group 2 (R_2) were given an injection of bone marrow in a dose of $2\cdot10^6$ cells. These mice were killed 2 h later, the spleens removed, a cell suspension prepared, and one-third of one spleen was injected into lethally irradiated recipients of group 3 (R_3). On the 9th day after transplantation the R_1 and R_3 mice were killed and the number of exogenous colonies counted. On average fewer than 0.2 endocolonies were formed in the spleen of the control mice without transplantation of hematopoietic cells. The f fraction was determined by the formula:

$$f = \frac{R_3 \cdot 3}{R_1 \cdot 40} \cdot 100,$$

where R_3 is the average number of colonies in the spleen of the R_3 mice, R_1 the average number of colonies in the spleen of the R_1 mice, and f the fraction of CFU-s of the transplant which migrated into the spleen and produced colonies (in percent).

In experiments to study specific inactivation of CFU-s by rabbit antiserum against *M. arthritidis* in the presence of complement, the antiserum was adsorbed beforehand by syngeneic bone marrow (37°C, 45 min). During the experiment the bone marrow cells were incubated first with the antiserum (4°C, 30 min), after which guinea pig complement was added (1:3, 37°C, 60 min), after which the number of CFU-s was determined in the exocolonization test.

EXPERIMENTAL RESULTS

In the experiments of series I the ability of *M. arthritidis* to stimulate emergence of CFU-s into the S phase of the cell cycle was studied in relation to the dose of mycoplasmas

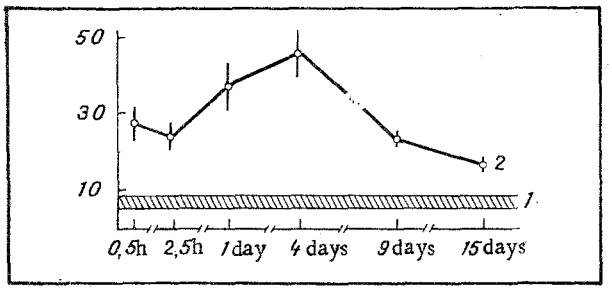

Fig. 1

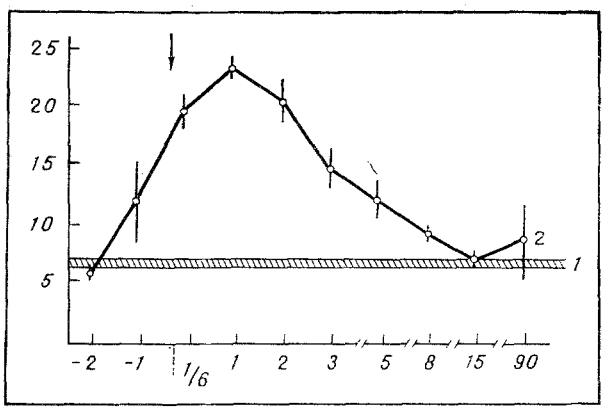

Fig. 2

Fig. 1. Number of CFU-s (in percent) of bone marrow of CBA mice infected with *M. arthritidis* in the S phase of the cell cycle. Abscissa, time after infection with *M. arthritidis*; ordinate, number of CFU-s in S phase (in percent). 1) Control, 2) *M. arthritidis*.

Fig. 2. Endogenous colony formation in spleen of sublethally irradiated BALB/c mice depending on time after injection with *M. arthritidis*. Abscissa, time of irradiation (in days) relative to time of infection with mycoplasma; ordinate, number of endogenous colonies in spleen. 1) Control; 2) number of colonies in spleen of infected mice. Arrow indicates time of infection with *M. arthritidis* in a dose of 10^8. Vertical lines show mean error.

used to infect CBA mice which, like BALB/c mice, are highly sensitive to *M. arthritidis*. As Table 1 shows, 24 h after infection of the mice, stimulation of emergence of CFU-s into the S phase was observed in animals receiving injections of mycoplasmas in doses of 10^7 and 10^8 CFU/ml. A smaller dose of the agent did not induce emergence of CFU-s into the cycle.

In the experiments of series II the duration of the states of increased proliferation of bone marrow CFU-s was studied after a single injection of *M. arthritidis* in a dose of 10^8 CFU/ml. As will be clear from Fig. 1, 30 min after injection of the mycoplasmas the number of CFU-s in the phase of DNA synthesis was considerably increased. This effect was more marked on the 1st and 4th days after infection, after which it gradually declined. Positive correlation was observed between the duration of stay of a large part of the CFU-s population in the cell cycle and the duration of the stimulating action of mycoplasmal infection on the formation of endogenous hematopoietic foci in the spleen of sublethally irradiated mice (Fig. 2).

In previous investigations [1, 2] no significant changes could be detected in the number of CFU-s in the bone marrow of mice infected with *M. arthritidis*, despite the marked stimulation of endocolonization of the spleen. It was tentatively suggested that under the influence of infection the affinity of CFU-s for the recipient's spleen may be disturbed, as a result of which the number of colonies determined in it was too low. Indirect evidence in support of this possibility was given by data on stimulation of emergence of CFU-s into the S phase, for it has been reported that the content of the f fraction may be lowered for proliferating CFU-s [11].

Testing this hypothesis showed (Table 2) that bone marrow CFU-s of mice infected with *M. arthritidis* 24 h before transplantation possess reduced ability to migrate into the spleen and to give rise to hematopoietic colonies. Of course, this effect could be due to a rise in the level of proliferation of CFU-s, but another, more interesting, hypothesis suggested itself: increased affinity of *M. arthritidis* for lympho-hematopoietic cells suggests that mycoplasmas, which are membrane parasites, on expressing themselves on the CFU-s membrane, can modify ecotaxis of the latter into the spleen. Actual confirmation of this possibility would be obtained by demonstrating the presence of mycoplasmas on the CFU-s membrane. Immunomorphologic methods are not very suitable for this purpose because of the extremely small number of CFU-s; consequently, a different experimental approach was chosen, based on demonstration of the cytotoxic action of antiserum against *M. arthritidis* on CFU-s in the bone marrow of infected mice.

These experiments showed that after transplantation of bone marrow of mice receiving an injection of *M. arthritidis* 24 h beforehand, the number of colonies was reduced on average by 47% after treatment of the transplant with specific antiserum in the presence of complement,

TABLE 3. Complement-Dependent Cytotoxic Effect of Specific Rabbit Antiserum on Transplantable Bone Marrow CFU-s of Mice Infected with *M. arthritidis*

No. of expt.	Donor of bone marrow	Treatment of cells	Number of colonies, M ± m	Number of mice	Degree of inactivation, percent
1	Normal	—	14,8±1,4	11	—
	»	NRS	14,3±1,2	12	3,4
	»	AMS	15,5±1,0	11	0 (—4,7)
	M. arthritidis	NRS	14,2±1,0	10	—
	The same	AMS	8,6±1,1*	10	39,4
2	Normal	NRS	10,6±1,5	9	—
	»	AMS	12,7±1,0	10	0 (—20)
	M. arthritidis	NRS	14,3±1,8	7	—
	The same	AMS	6,6±1,3*	9	54,2

*P < 0.05 compared with control.
Legend. NRS) normal rabbit serum, AMS) antimycoplasmal serum.

but was unchanged if the transplant was incubated with normal rabbit serum (Table 3). Treatment of bone marrow of intact mice both with antiserum and with normal serum in the presence of complement did not affect the number of colonies. These results are evidence of possible binding of the mycoplasmas with the CFU-s membrane.

The results as a whole are thus evidence that a single injection of *M. arthritidis* has a marked effect on the hematopoietic system of mice. Many CFU-s, even in the early stage after infection, emerge into the S phase of the cell cycle, and the state of increased proliferative activity lasts at least 2 weeks. Bacterial endotoxins [3, 9, 12], antigens of salmonellas [6], and *C. parvum* [8], and other agents also have a similar action on CFU-s. However, bacterial endotoxins are characterized by a rapidly induced, intensive release of CFU-s from the bone marrow, on account of which the level of circulating CFU-s and their concentration in the spleen rise sharply [7, 12]. Conversely, after a single injection of *C. parvum* the number of CFU-s in the bone marrow increased [10], but after infection with *M. arthritidis* it did not change significantly [1, 2]. However, after a study of the test fraction of bone marrow CFU-s of mice injected with different agents, the complexity of these views increased: it was found that inoculation of endotoxins leads to a decrease in the f fraction [7]. The same effect, to judge from the data obtained in the present investigation, is produced by *M. arthritidis* also. If this is so, the true number of CFU-s in the bone marrow of infected mice increases, and in subsequent experiments a correction must be introduced to the value of f. Ecotaxis of CFU-s into the spleen is perhaps modified because of the presence of mycoplasmas on the CFU-s membrane, for if antigen-receptor characteristics of CFU-s are disturbed, this could totally alter the homing effect. This hypothesis requires experimental verification.

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