LITERATURE CITED

1. B. Hoffman and P. Cranefield, Electrophysiology of the Heart, McGraw-Hill (1960).
2. M. Schaldach and S. Furman, Advances in Pacemaker Technology, Berlin (1975).
3. H. Thalen et al., The Artificial Cardiac Pacemaker, Springfield (1969).

ERYTHROPOIETIN-INDEPENDENT STIMULATION OF ERYTHROPOIESIS IN MICE INFECTED WITH *Mycoplasma arthritidis*

A. V. Sanin, V. V. Khorobrykh,
and D. R. Kaulen

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Previously [2] we reported the ability of *Mycoplasma arthritidis* to stimulate endogenous colony formation in sublethally irradiated mice. A subsequent study of the stimulation of hematopoiesis induced by mycoplasmas suggested that *M. arthritidis* may perhaps act not on hematopoietic stem cells, but on already committed precursor cells, most likely of the erythroid series [3].

The object of this investigation was to attempt to shed further light on this problem.

EXPERIMENTAL METHOD

BALB/c and (C57BL/6 × A/Sn)F_1(BAF_1) mice aged 8-10 weeks were obtained from the Rappolovo nursery, Academy of Medical Sciences of the USSR.

The mycoplasmas were obtained as described previously [1] and kept at −70°C. Mice were infected intraperitoneally with a dose of 0.5 ml of mycoplasmas with a titer of 2×10^8 colony-forming units/ml. Nutrient medium for growth of the mycoplasmas was injected into control mice.

Plethora was created by the method of Curray et al. [4]. Blood of heparinized donors was washed twice and the erythrocytes resuspended in sterile physiological saline. A 60% erythrocyte suspension was injected intraperitoneally into the mice in a dose of 1 ml 4 and 2 days or 3 and 1 days before irradiation. The mice were irradiated in a dose of 550 and 920 rads. Hematopoietic cells were cloned *in vivo* in lethally irradiated mice by the method of Till and McCulloch [6]. The mice were killed on the 7th-9th day after irradiation, the spleens were fixed in Bouin's solution, and the number of visible colonies was counted macroscopically after 4 h.

The appearance of endogenous erythropoietin (EP) in intact BAF_1 mice and in mice infected with mycoplasmas 3 and 1 days before sacrifice was tested by the use of blood plasma which was inactivated (30 min at 56°C) and kept at −20°C. The test plasma was injected into mice with plethora 1, 3, and 4 days after the second transfusion, and on the 5th day 0.5 μCi ^{59}Fe in 0.5 ml physiological saline was injected intravenously. The mice were killed 24 h after injection of the isotope. Radioactivity in the spleen and blood was counted on a Nuclear Chicago Gamma-Counter and expressed relative to the activity injected. The blood volume was taken to be 5% of body weight. Plasma from anemic mice, in which blood loss was produced 48 and 24 h before removal of the plasma by taking 0.4-0.5 ml of blood from the anterior chamber of the eye, was used as the source of exogenous EP.

EXPERIMENTAL RESULTS

In the experiments of series I the effect of *M. arthritidis* on endogenous colony

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TABLE 1. Effect of *Mycoplasma arthritidis* on Endogenous Colony Formation in Mice with Plethora and Normal Mice Irradiated in a Dose of 550 rads

Variant of experiment	Mice of line (C57BL/6×A/Sn)F_1					Mice of line BALB/c				
	plethora	time (in h) of infection with mycoplasmas relative to irradiation			number of endogenous colonies (M ± m)	plethora	time (in h) of infection with mycoplasmas relative to irradiation			number of endogenous colonies (M ± m)
		−24	4	48			−24	24	48	
1	+	−	−	−	0,8±0,3 (0,2—1,4)	+	−	−	−	0,4±0,3 (0—1,0)
2	+	−	−	+	10,6±1,4 (7,1—14,1)	+	−	+	+	14,5±3,2 (7,5—21,3)
3	+	+	+	+	29,6±6,6 (15,5—43,7)	+	+	+	+	37,0±9,5 (17,0—57,0)
4	+	+	−	−	16,4±1,6 (11,8—21,0)	+	+	−	−	15,6±1,4 (12,2—19,0)
5	−	−	−	−	12,4±1,0 (10,4—14,4)	−	−	−	−	10,9±1,2 (8,4—13,4)
6	−	−	+	−	23,8±2,2 (19,0—28,6)	−	−	+	−	16,8±3,6 (13,2—20,4)
7	−	+	−	−	38,6±4,5 (26,4—48,8)	−	+	−	−	39,7±8,9 (20,8±58,6)

Legend. Time of irradiation taken as 0 h; confidence interval calculated at P = 0.05 level given in parentheses.

formation was studied in mice with plethora. As Table 1 shows, injection of the mycoplasma 24 h before sublethal irradiation or 4-48 h thereafter completely restored the number of endogenous foci to that discovered in intact mice (variant 5). Three injections of mycoplasmas (variant 3) led to a significant increase in endogenous colony formation comparable with that observed in mice with plethora and infected with mycoplasmas 24 h before irradiation (variant 7). Practically no endogenous colonies are known to develop in irradiated mice with plethora. This is explained on the grounds that endogenous colonies on the surface of the spleen are mainly erythroid in nature, and synthesis of endogenous EP is temporarily blocked in plethora [4]. In the present experiments, endogenous colony formation was sharply depressed in the irradiated mice with plethora (variant 1), but injection of mycoplasmas led to simulation of endogenous colony formation (variants 2-4), mainly erythroid.

It can be concluded from these results that mycoplasmas restore erythropoiesis in mice with plethora. This could happen if one of the following conditions was satisfied: Either *M. arthritidis* acts on cells synthesizing EP in such a way that blocking of EP synthesis is abolished and the newly formed hormone stimulates erythroid differentiation or the mycoplasma acts directly on erythroid precursor cells, stimulating their subsequent proliferation and differentiation independently of EP.

To test whether the endogenous EP level is raised in mice infected with *M. arthritidis*, the known test system for detection of endogenous EP [7] was used. As will be clear from Table 2, no increase was found in EP activity in plasma taken from the mice 1 or 3 days after infection with mycoplasmas: Incorporation of ^{59}Fe in the blood and spleen of mice inoculated with the test preparations was the same as in control mice with plethora. Plasma of mice with anemia (the source of exogenous EP) promoted recovery of erythropoiesis to the normal level. Cells of *M. arthritidis* themselves, injected into mice with plethora, led to significant stimulation of erythropoiesis in the spleen (2.3% compared with 1.1% in the control) with no increase in incorporation of ^{59}Fe into the blood.

The same samples of plasma were subjected to parallel tests in a different test system (series II of experiments): BAF_1 mice with plethora were irradiated in a lethal dose (920 rads), they were then given an injection of 3.5×10^4 bone marrow cells from a syngeneic intact donor, and for 3 successive days they then received injections of 0.25 ml of the plasma to be tested for the presence of EP. After injection of plasma of normal mice and plasma of mice infected with mycoplasmas, the number of exogenous foci which developed in the spleen of recipients of the bone marrow cells was 2.3 + 0.6 and 2.3 + 0.7, respectively, whereas in mice without plethora receiving the same number of cells, the number of foci was 15.4 + 1.7.

No increase in endogenous EP synthesis in mice infected with mycoplasmas could thus be

TABLE 2. Determination of Endogenous EP in Plasma of BAF_1 Mice Infected with *M. arthritidis* (M ± m)

Plethora	Factor tested	Blood		Spleen	
		cpm	%	cpm	%
++	—	139,3±21,1	1,1	328,3±52,1	1,1
++	Plasma N	150,9±14,8	1,2	315,0±23,2	1,1
++	Plasma M (1st day)	127,0±14,5	0,9	279,4±25,1	1,0
++	Plasma M (3rd day)	219,2±51,6	1,7	336,0±40,1	1,1
++	Mycoplasma	136,8±15,3	1,1	678,8±71,7	2,3*
+	EP	3463,5±172,7	29,7	2088,0±322,9	6,9
—	N	4007,0±157,2	30,5	1384,6±144,4	4,9

*Difference from control significant ($P < 0.05$).

Legend. Incorporation of ^{59}Fe shown as a percentage of total injected activity. N) Normal mice; M) mice infected with mycoplasmas.

found by the test system used. If these methods are sufficiently sensitive it can be postulated that stimulation of erythropoiesis, induced by *M. arthritidis*, in mice with plethora is independent of EP. Convincing evidence has now been obtained to show that production of early erythroid precursors is independent of EP and is not disturbed during plethora [5]. Evidently *M. arthritidis* acts on the least differentiated erythroid cells, "triggering" them into proliferation in the absence of EP.

LITERATURE CITED

1. G. Ya. Kagan, I. V. Rakovskaya, T. D. Morgunova, et al., Vopr. Virusol., No. 2, 171 (1975).
2. A. V. Sanin, D. R. Kaulen, V. V. Khorobrykh, et al., Byull. Éksp. Biol. Med., No. 8, 179 (1979).
3. A. V. Sanin, V. V. Khorobrykh, A. V. Pronin, et al., Nauch. Dokl. Vyssh. Shkoly, Biol. Nauki, No. 4, 95 (1980).
4. J. L. Curry, J. J. Trentin, and N. Wolf, J. Exp. Med., 125, 703 (1967).
5. N. N. Iscove and L. J. Guilbert, in: *In vitro* Aspects of Erythropoiesis, M. J. Murphy, ed., New York (1978), pp. 3-7.
6. J. E. Till and E. A. McCulloch, Radiat. Res., 14, 213 (1961).
7. D. Van Dyke, M. Lou Nohr, and J. H. Lawrence, Blood, 28, 535 (1966).