# STIMULATION OF ERYTHROPOIESIS IN MICE WITH MYCOPLASMA INFECTION

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It was shown previously that *Mycoplasma arthritidis* stimulates endogenous colony formation in the spleen of sublethally irradiated mice [3]. However, the number of hemopoietic stem cells in the bone marrow and spleen of the mice was not increased in mycoplasma infection, as was found after transplantation of a suspension of hematopoietic cells into mice irradiated with a lethal dose [5]. It was later shown that *M. arthritidis* stimulates erythropoiesis in plethoric mice, and the stimulation effect is evidently independent of erythropoietin (EP) [4].

The object of the present investigation was to continue the study of the action of mycoplasmas on erythropoiesis.

## EXPERIMENTAL METHOD

(C57BL/6 × A/Sn)$F_1$ mice of both sexes, weighing 21-23 g, obtained from the "Rappolovo" nursery, Academy of Medical Sciences of the USSR, were used.

*M. arthritidis* and *A. laidlawii* were obtained as described previously [1, 2]. Plasma of anemic mice, from the anterior chamber of whose eye 0.5 ml blood was taken 1 and 2 days before collection of the plasma, was used as the source of exogenous EP.

Plethora was produced by the method of Curry et al. [6]. The mice were infected 24 h after the second transfusion of a 60% suspension of homologous mouse erythrocytes with *M. arthritidis* which was injected intraperitoneally in a dose of 0.5 ml. $^{59}$Fe (ferrous citrate with a specific activity of 0.2 mCi/ml) was injected intravenously in a dose of 0.5 μCi in 0.5 ml physiological saline, exactly 24 h before sacrifice of the mice each time. Radioactivity in the blood and spleen of the mice was measured on a Gamma Counter (Nuclear Chicago). The percentage of incorporation of label was determined relative to injected activity. The blood volume of the plethoric mice was taken to be 8.7% of body weight [14].

To inhibit erythropoiesis without disturbing synthesis of endogenous EP, the method of Reissman and Ito [13] in our modification was used: actinomycin D (from Serva, West Germany) was dissolved in triple distilled water to a concentration of 16 μg/ml and injected subcutaneously daily for five days in a dose of 0.1 ml into the mice; 1 h after the last injection, the mice were irradiated in a sublethal dose (620 rads) and the test preparations were injected 1 h later.

The significance of differences between the results was assessed by Student's t-test.

## EXPERIMENTAL RESULTS

The study of the dynamics of incorporation of $^{59}$Fe into the blood of the plethoric mice infected with *M. arthritidis* revealed no significant changes compared with the control (Fig. 1A). However, a significant increase in incorporation of radioactive iron was observed in the spleen of the infected mice on the 4th day after infection (Fig. 1B). On the 5th day the stimulating effect still remained, but on the 7th day incorporation of $^{59}$Fe into the erythroid cells of their spleen was practically the same as in control mice.

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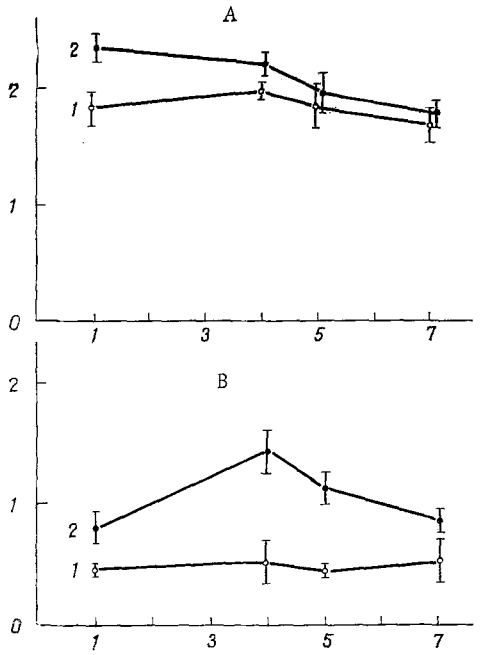


Fig. 1. Incorporation of $^{59}Fe$ into blood (A) and spleen (B) of mice. Abscissa, days after infection; ordinate, percentage incorporation of $^{59}Fe$. Vertical lines denote standard error of arithmetic means. 1) Incorporation of $^{59}Fe$ in control mice, 2) the same, in infected mice.

TABLE 1. Effect of Actinomycin D on Endogenous Colony Formation in Spleen of $BAF_1$ Mice in Response to Injection of Various Agents

| Group of mice | Number of mice | Number of endogenous colonies (M ± m) | | |
|---|---|---|---|---|
| | | 620 rads | number of mice | actinomycin D + 620 rads |
| Control | 21 | 8,6±0,8 (6,9—10,3) | 12<br>8 | 2,3±0,6 (1,0—3,6) |
| Erythropoietin | 12 | 21,0±4,1 (12,3—29,7) | 8 | 3,4±0,8 (1,7—5,1) |
| M. arthritidis | 8 | 16,1±2,5 (10,8—21,4) | 8 | 13,6±1,8 (9,8—17,4) |
| A. laidlawii | 16 | 14,9±1,7 (11,3—18,5) | 9 | 13,3±3,1 (6,7—19,9) |

Legend. Confidence interval calculated at $\overline{P = 0.05}$ level given in parentheses.

To continue the study of this phenomenon, the experimental system described by Reissmann and Ito [13] based on repeated injections of small doses of actinomycin D into mice, was followed. Under these circumstances erythropoiesis was completely blocked although synthesis of endogenous EP was undisturbed. After reproducing this system, the mice were irradiated in a sublethal dose and *M. arthritidis* or *A. laidlawii*, or anemic mouse plasma with an increased EP concentration was injected. Control mice did not receive actinomycin D. Injection of actinomycin D before sublethal irradiation was found to inhibit endogenous colony formation: On average 2.3 endogenous foci developed in the spleen of mice inoculated with actinomycin D, whereas the mean number of endocolonies in mice not receiving actinomycin D was 8.6 (Table 1). Exogenous EP, injection of which into the control mice led to stimulation of endogenous colony formation, did not increase the number of such colonies in mice receiving actinomycin D. Conversely, both *M. arthritidis* and *A. laidlawii* completely

abolished the effect of actinomycin D, and produced the same stimulation of endogenous colony formation as in mice not receiving actinomycin D. This last fact confirms previous observations [4] that mycoplasma infection leads to erythropoietin-independent stimulation of erythropoiesis; injection of exogenous EP into mice inoculated with actinomycin D was ineffective (Table 1), in agreement with data obtained by other workers [13].

The mechanism of action of actinomycin D on erythropoietin was blocking of differentiation of what, according to recent information [9, 11], is the youngest erythroid precursor, the so-called burst-forming unit ($BFU_e$) into a cell that gives rise to an erythroid colony in vitro ($CFU_e$) [16]. After the third injection of actinomycin D, practically no $CFU_e$ could be detected [15], a fact with which loss of ability of the erythroid system to "respond" to additional injection of EP is evidently connected. Evidently *M. arthritidis* acts on less highly differentiated cells than $CFU_e$; evidence of this is also given by the ability of this mycoplasma to stimulate erythropoiesis in plethoric mice [4], in which the number of $CFU_e$ is several times smaller [10]. It is also known that the earliest stages of formation of erythroid precursors are characterized by independence of EP [10, 12]. From this point of view, immature erythroid cells, standing at about the level of $BFU_e$, and in particular, those cells that so far have received little study but which have been called "colony-forming units giving rise to transient erythroid colonies" ($CFU_{te}$) [8], may be the targets for mycoplasmas. The formation of $CFU_{te}$ is undisturbed by plethora [8].

The problem of the nature of the target cells for *M. arthritidis*, which is capable of activating the leukemogenic potential of mouse leukemia viruses [1, 2, 7], is important to our understanding of the mechanism of the coleukemogenic action and pathogenicity of this species of mycoplasma, and it will be a topic for future study by the writers.

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