tional properties. In that case they behave as helpers (T, T_{act}) or as depressors (T_{rab}, B + "null"), but they may also possess both types of activity (B_{mouse}). In relation to B_{mouse} lymphocytes this depends on the initial level of the reactions: Inhibition is characteristic of a value of 13.5-1.6%, stimulation is characteristic of a value of 7.3-2.3% ($P < 0.05$). Lymphocytes may have a corresponding effect on phagocyte function in an inflammatory focus, where the proportions of the various mononuclear subpopulations may differ from the peripheral blood picture: In some patients with suppurative wound infection the number of B_{mouse}, T_{rab} and, in particular, of T_{act} cells is increased.

The results offer prospects of a new method of assessing the role of lymphocytes in infection through their regulating influence on the (phagocytes—basic effects of antibacterial immunity) system.

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STIMULATION OF TRANSIENT ENDOGENOUS SPLENIC COLONY FORMATION IN NORMAL AND PLETHORIC MICE INFECTED WITH *Mycoplasma arthritidis*

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KEY WORDS: transient endogenous colonies; plethora; *Mycoplasma arthritidis*.

Features distinguishing the action of *Mycoplasma arthritidis* on the hematopoietic system of experimentally infected mice include stimulation of endogenous colony formation [3], activation of erythropoiesis in sublethally irradiated [4] plethoric [1, 5] mice, and abolition of the block of erythroid differentiation induced by repeated injections of small doses of actinomycin D, which is evidence of the erythropoietin-independent character of the stimulating effect of this organism [1], have led to the hypothesis that the target cells for *M. arthritidis* may be found among precursors at the stage(s) of differentiation between colony-forming stem cells and erythroid cells, production of which from precursors depends on erythropoietin (EP). Colony-forming cells forming transient endogenous colonies in the spleen (CFU-TE) satisfy these conditions. They begin to appear on the 3rd day after sublethal irradiation; on the 7th day the colonies disappear [6]. CFU-TE production from hemat-

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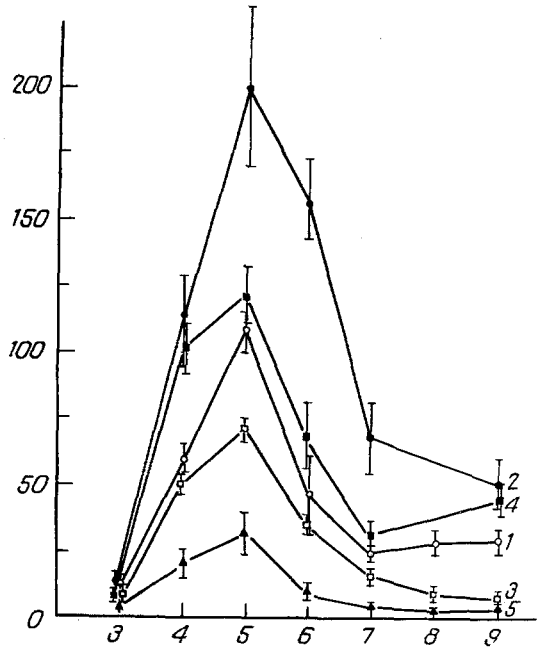

Fig. 1. Time course of formation of endogenous colonies in normal and infected mice depending on dose of irradiation. 1, 3, 5) Control, irradiation in doses of 4, 5, and 6 Gy respectively; 2, 4) infection with *M. arthritidis* 24 h before irradiation in doses of 4 and 5 Gy respectively. Abscissa, time (in days) after irradiation; ordinate, number of endogenous colonies in spleen.

TABLE 1. Transient Endogenous Splenic Colony Formation in Plethoric CBA Mice Irradiated in a Dose of 4 Gy (M ± m)

Inoculum	Time after irradiation, days	
	5	6
Control	11,0±1,4 (7,8+14,2)	5,0±1,1 (2,2—7,8)
M. arthritidis	Over 150 (confluent growth)	52,4±6,9 (34,8—70,0)
Serum of anemic mice	24,0±3,6 (confluent growth)	38,8±11,2 (7,6—70,0)

Legend. Confidence interval shown in parentheses (P = 0.05).

opoietic stem cells is undisturbed in plethora, i.e., it is independent of EP although subsequent differentiation is sensitive to EP [8] and is stimulated by blood loss [6, 8] or by exogenous EP [8].

The aim of this investigation was to study the action of *M. arthritidis* on CFU-TE.

EXPERIMENTAL METHOD

Experiments were carried out on male CBA mice weighing 20-22 g, obtained from the "Stolbovaya" Nursery, Academy of Medical Sciences of the USSR, and on $B6AF_1$ $(C57BL/6 \times A/Sn)F_1$ mice of both sexes obtained from the "Rappolovo" Nursery, Academy of Medical Sciences of the USSR.

The mycoplasmas were obtained as described previously [2]. Mice were infected intraperitoneally with *M. arthritidis* in a dose of 10^8 colony-forming units.

TABLE 2. Incorporation of ^{59}Fe (in % of injected radioactivity) in Bone Marrow and Spleen of Plethoric CBA Mice Irradiated in a Dose of 4 Gy (M ± m)

Object	Inoculum	Time after irradiation, days		
		4	5	6
Bone marrow	Control	0,52 ± 0,07	0,60 ± 0,03	0,47 ± 0,04
	M. arthritidis	0,40 ± 0,08	0,48 ± 0,05	0,49 ± 0,05
	Serum of anemic mice	0,70 ± 0,03	0,86 ± 0,04	0,92 ± 0,06
Spleen	Control	0,44 ± 0,04	0,51 ± 0,08	0,44 ± 0,10
	M. arthritidis	0,71 ± 0,15	0,71 ± 0,09	0,69 ± 0,01
	Serum of anemic mice	0,70 ± 0,09	0,62 ± 0,14	0,61 ± 0,12

CFU-TE were detected by counting colonies of hematopoietic cells under a magnifying glass on the surface of spleens fixed in Bouin's solution at different times after irradiation in a sublethal dose (4.0-6.0 Gy).

Plethora was restored by the method of Curry et al. [7]. Mycoplasmas were injected 24 h after the second transfusion of a suspension of isologous erythrocytes.

^{59}Fe (ferrous citrate, specific activity 0.2 mCi/ml) was injected intraperitoneally in a dose of 0.5 μCi in 0.5 ml physiological saline 6 h before sacrifice of the mice. Radioactivity in the spleen and bone marrow was determined on a Gamma spectrometer (Nuclear Chicago, USA).

EXPERIMENTAL RESULTS

The time course of formation of transient endogenous splenic colonies in the mice depending on the dose of irradiation is illustrated in Fig. 1. With a decrease in the dose from 6 Gy to 5 and 4 Gy the number of colonies increased; their formation reached a maximum on the 5th day, in agreement with observations by other workers [8, 10, 11]. Injection of *M. arthritidis* into CBA mice 24 h before irradiation led to marked stimulation of formation of transient colonies, and also of colonies detectable on the 9th day (arising from hematopoietic stem cells). Identical results were obtained in experiments with mice of the other genotype ($B6AF_1$).

In plethoric CBA mice irradiated in a dose of 4 Gy, transient colony formation was depressed (Table 1): The number of colonies at all times was reduced about tenfold compared with their number in the spleen of normal mice. However, plethora had virtually no effect on the development of transient colonies in the spleen of mice infected with *M. arthritidis*: Their formation was greatly enhanced, evidence that the activating action of *M. arthritidis* on CFU-TE is EP-independent. Serum with an increased EP content obtained from anemic (after blood loss) mice also stimulated transient endogenous colony formation in the spleen of plethoric mice, but the effect was weaker than that of *M. arthritidis*. Such great differences between the control and experiment as were observed in the number of transient colonies at the same times (4th-6th days) after irradiation could not be detected by measuring ^{59}Fe incorporation into heme of erythroid cells in the spleen and bone marrow (Table 2). This was evidently connected with the fact that CFU-TE give rise not only to erythroid, but also to megakaryocytic and granulocytic colonies [11], which do not take up ^{59}Fe.

A study of the dynamics of ^{59}Fe incorporation into erythroid cells in the bone marrow of normal (not plethoric) hybrid $B6AF_1$ mice irradiated in a dose of 6 Gy showed (Fig. 2A) a transient peak on the 6th day after irradiation in the regenerating bone marrow of mice infected with *M. arthritidis*. A similar peak of ^{59}Fe incorporation could be found in the spleen of normal and infected mice on the 5th-6th day after irradiation (Fig. 2B), although its value varied from one experiment to another, possibly due to the low sensitivity of the method. After this transient peak, on the 8th-10th day after irradiation, the main peak of isotope incorporation corresponding to the development of endogenous colonies arising from hematopoietic stem cells appeared.

The results described above thus indicate that *M. arthritidis* may have a stimulating action on the CFU-TE population in the spleen (and also, perhaps, in bone marrow, see Fig. 2A). These cells, first found by Robinson [10], are evidently a special reserve category (possibly

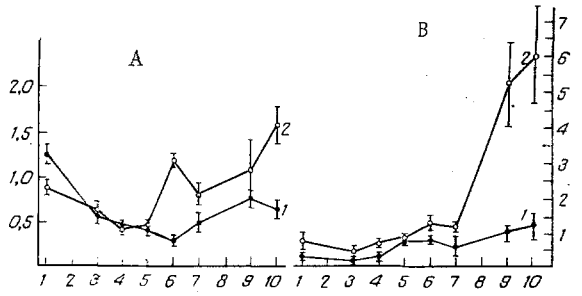

Fig. 2. Dynamics of ^{59}Fe incorporation in bone marrow (A) and spleen (B) of B6AF$_1$ mice irradiated in a dose of 6 Gy. 1) Control; 2) mice infected with *M. arthritidis* 24 h before irradiation. Abscissa, time (in days) after irradiation; ordinate, incorporation of ^{59}Fe (in %).

a side branch) of hematopoietic precursors, which are activated when hematopoiesis is under stress [8]. As the progeny of hematopoietic stem cells CFU-TE have progressed considerably further along the histogenetic line of development, but still preserve their competence for differentiation in the direction of erythroid, megakaryocytic, and granulocytic-monocytic branches of hematopoiesis [11]. The biological significance of the existence of such a reserve compartment of hematopoietic precursors will be evident: There is an urgent need for mature cells after exposure to a stress-inducing factor such as irradiation. For hematopoietic stem cells surviving after irradiation to commence differentiation, a certain lag period must be gone through, during which the stem cells proliferate until they reach a threshold number, after which differentiation begins [6]. The CFU-TE set out on "suicidal" differentiation a few days earlier, and colonies formed by the progeny of these cells are "absorbed" on the 7th day after irradiation, having satisfied the need of the organism for mature cells. Data on the effect of mycoplasmas, for which not only animals but also man act as carriers, on CFU-TE are important in this connection. The possibility cannot be ruled out that this "triggering" of CFU-TE may explain the ability of *M. arthritidis* to sharply enhance the postradiation survival rate of mice irradiated in a lethal dose [2]. An adverse manifestation of this phenomenon may be an increase in the number of hematopoietic precursors which are target cells for leukemogenic viruses, which in the case of a mixed mycoplasma—virus carrier state is the cause of induction of leukemia in resistant mice [2, 3, 4]. Hitherto proliferation of CFU-TE has been stimulated either by blood loss a few hours after irradiation [6, 8, 11] or by injection of EP [8], i.e., by creating an increased demand for erythroid differentiation. In plethora, on the other hand, when endogenous EP production has temporarily ceased [7], the formation of transient endogenous colonies is depressed [8, 11], although production of CFU-TE from stem cells is not disturbed [8]. We know [9] that repeated injections of small doses of actinomycin D lead to specific blocking of erythropoiesis on account of temporary cessation of differentiation of erythroid burst-forming units (BFUe) in the progeny more sensitive to EP [12]. However, injection of *M. arthritidis* abolishes this block [1] and also, as the present investigation demonstrated, triggers differentiation of CFU-TE in the same way as EP, but independently of it, for EP production is not increased in mice infected with *M. arthritidis* [5].

It can be postulated on the basis of all the facts described above that CFU-TE (and also, possibly, BFUe and other precursors whose production is not disturbed in plethora) may act as target cells for the action of *M. arthritidis*. The predominant activation of these cells may play a key role in the mechanism of the hematopoietic disorders and disturbance of the formation of an effective immune response in infections with mycoplasmas and combined infections with mycoplasmas and viruses.

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ISOLATION OF HYBRID HUMAN–RODENT SOMATIC CELL CLONES

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KEY WORDS: hybrid cells; man; mouse; Chinese hamster.

Hybridization of mammalian somatic cells is widely used in the investigation of differentiation, of regulation of gene action, and chromosome mapping. The most popular method of obtaining hybrid cells is by fusion of cells of two mutant lines and growth of the cells on a selective medium [1]. Definite difficulties arise in the production of hybrid cells when normal nonmutant human fibroblasts are used as one of the partners.

In the investigation described below a selective system was worked out for obtaining hybrids by fusing normal human cells growing on a substrate with mutant Chinese hamster and mouse cells.

EXPERIMENTAL METHOD

Mutant Chinese hamster cells (M) resistant to 30 μg/ml of 6-mercaptopurine, clone M01 isolated from M cells after culture on medium with ouabain (1 mM), normal human embryonic skin fibroblasts of strains F20 and IMG795, normal human embryonic muscle cells of strain IMG812, mutant LTK mouse cells resistant to 30 μg/ml of 5-bromodeoxyuridine, and clone L01, obtained after culturing LTK cells on medium with ouabain (1 mM) were used. The cells were cultured in Carrel flasks on Eagle's medium with the addition of 20% bovine serum without antibiotics; this medium will be described later as normal medium.

The components of selective medium HATG3 were hypoxanthine (from Chemapol, Czechoslovakia) 10^{-4} M, aminopterin (from Schuchardt, West Germany) $4 \cdot 10^{-7}$ M, thimidine (from Serva, West Germany) $1.6 \cdot 10^{-7}$ M, and glycine (from Reanal, Hungary) $3 \cdot 10^{-6}$ M.

Ouabain (from Serva, West Germany) was used in the concentrations indicated below. The experiments were conducted on plastic petri dishes (diameter 40 mm, Leningrad Factory) or glass dishes (diameter 60 and 100 mm, from Anumbra, Czechoslovakia). To maintain the pH of the medium between 7.2 and 7.4 the dishes were kept in airtight containers under an atmosphere containing 5% CO_2. To count the growing colonies the cells were stained with methylene blue. To count the number of growing colonies, nuclei were stained with azure-eosin. Films for karyologic analysis were prepared with cells harvested by shaking the Carrel flasks. The films were air-dried by the usual method and stained with 2% Giemsa stain by the G method, using trypsin or CsCl. At least 10 metaphase plates were analyzed in each clone.

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