Indirect visualization of hematopoietic colony-forming stem cell (CFUs) as a possible target cell for *Mycoplasma arthritidis*

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Summary. Utilizing specific rabbit antiserum against *M. arthritidis* together with complement, a portion of the marrow 7-day CFUs population of CBA mice infected with live mycoplasma organisms 1 day previously was shown to be inactivated. These cells might therefore be considered as candidate target cells for *M. arthritidis*. 11-day CFUs were unaffected by similar treatment.
Key words. Mycoplasma arthritidis; CFUs; target cells.

Previous studies in this laboratory and elsewhere have shown that *M. arthritidis* (a membrane-associated organism, causative agent of arthritic disease in murine hosts) may cause a severe disturbance of the hematopoietic system in mice[1–4]. Injection of live mycoplasma organisms has been shown to augment endogenous colony formation in the spleen[1], and to increase the proliferative rate of transient endogenous colony-forming cells (TE-CFU)[2]. Other studies concerned with the erythroid differentiation of CFUs have shown that anemia development[3], and a wave of erythropoietin-independent erythropoiesis, are induced by *M. arthritidis* in plethoric and actinomycin-D-treated mice[4]. Dual injection of *M. arthritidis* and Rauscher murine leukemia virus (R-MuLV) into resistant (C57BL/6 × A/He)F_1 hybrid mice led to the erythroblastosis development in the majority of the mice[5], suggesting that hematopoietic target cells for R-MuLV (CFUs or primitive erythroid precursors[6]) were directly activated by mycoplasma organisms. In order to obtain further evidence for the possible involvement of the CFU compartment in *M. arthritidis*-induced hematopoietic disorder, additional experiments were carried out. Using an indirect approach, an attempt was made to reveal whether any of the bone marrow CFUs of *M. arthritidis*-infected mice might carry the membrane-associated mycoplasmas and therefore be sensitive to complement-dependent cytotoxic action of the specific antiserum.

Materials and methods. Specific rabbit antiserum to *M. arthritidis* PG6 (obtained from Prof. E. A. Freundt, Aarhus University, Denmark) was pooled and frozen until used.

Adult male CBA mice, weighing 18–20 g, were challenged with a single i.p. injection of 20×10^7 live mycoplasma organisms. One day thereafter groups of three normal (saline-treated) and three *M. arthritidis*-infected mice were sacrificed by cervical dislocation and pooled bone marrow cell suspensions were prepared in cold Hank's BSS. Three equal aliquots of 1 ml vol. containing approximately 10×10^6 marrow cells were prepared from this suspension. To each one of these were added 1 ml of antiserum (diluted 1:2) that had previously been absorbed with normal CBA bone marrow cells for 45 min at 37 °C, or normal rabbit serum (absorbed as above to avoid possible cytotoxicity), or Hank's solution. The suspensions were then incubated at 4 °C for 60 min. At the end of that time 0.5 ml of fresh guinea pig complement (1:5) was added and the suspensions were further incubated at 37 °C for 30 min.

Table 1. Cytotoxic effect of rabbit anti-*M. arthritidis* antiserum on the 7-day bone marrow CFUs of normal and *M. arthritidis*-infected CBA mice, compared to the effect of suspension medium and normal rabbit serum

Incubation medium	CFUs/5 × 10^4 bone marrow cells	
	Normal bone marrow cells	Bone marrow cells of *M. arthritidis*-infected mice
Hank's BSS	12.9 ± 1.0[a]	13.3 ± 1.0
Normal rabbit serum	12.7 ± 1.1	14.2 ± 1.0
Antiserum	14.2 ± 0.8	7.6 ± 0.8
Inhibition of colony-formation by antiserum (%)	None	46.5

[a] Mean ± 1 SE of 20 or more separate determinations. Data from 2 experiments yielding practically identical results are summarized.

The assay for colony-forming ability was carried out using the Till and McCulloch exocolonization technique[7]. Suspensions of the antiserum-treated and control cells were injected via the lateral tail vein into syngeneic CBA mice that had received 8.0 Gy of X-irradiation 2 h previously. 7 or 11–12 days later these recipients were sacrificed and their spleens removed and fixed in Bouin's solution. The superficial colonies were counted, and a comparison between the cells treated with antiserum and those treated with normal serum or Hank's BSS made. Background counts in simultaneously irradiated, non-injected control mice were negligible; less than one colony in 10 spleens.

Assay for seeding efficiency ('f' fraction) of transplanted hematopoietic cells was carried out acccording to Siminovitch et al.[8].

Results. Relative to the number of CFUs per 5×10^4 injected marrow cells, there was no difference in the number of 7-day colonies formed when incubation in suspension medium was compared with that in normal rabbit serum (table 1). However, specific rabbit antiserum proved to be highly cytotoxic for the bone marrow 7-day CFUs of *M. arthritidis*-treated donor mice. The degree of colony-forming ability inactivation was approximately 2-fold. Since normal rabbit serum absorbed with normal CBA marrow cells did not eradicate CFUs, the inactivation of the latter by the anti-*M. arthritidis* antiserum could be ascribed to the specific action of this antiserum. When assayed at days 11–12, no significant differences were observed in the numbers of spleen colonies in any recipient mice that had been injected with normal or treated with either of the sera bone marrow cells (data not shown).

The 2-h spleen-seeding efficiencies of 7-day and 12-day CFUs in normal bone marrow, determined as described[8], were 0.15 and 0.10, respectively (table 2). The corresponding values for the bone marrow 7-day and 12-day CFUs of *M. arthritidis*-infected mice were 0.06 and 0.10.

Discussion. The findings of this study provide additional evidence suggesting that at least a subpopulation of CFUs should be considered as candidate target cells for *Mycoplasma arthritidis*. Murine hematopoietic stem cells have generally been assayed by their ability to form macroscopic spleen colonies in the recipient mice 7–9 days after irradiation[7]. Magli et al.[9] reported the transient nature of 7–8-day colony-forming cells and suggested that only when colonies are scored at or later than 11–12 days is the CFUs assay adequate for the measure of pluripotent stem cells. An antigenic difference was reported between subpopulations of CFUs forming early (8-day) and late (14-day) spleen colonies, that was due to progressive loss of Qa-m2 expression with differentiation from day 14 CFUs to day 8 CFUs[10]. Here specific rabbit antiserum was found to inactivate half of the 7-day CFUs bone marrow population of *M. arthritidis*-infected mice, while having no effect on the CFUs forming

Table 2. Loss of transplantation efficiency of exogenous CFUs derived from bone marrow 1 day following *M. arthritidis* injection

Mice	2-h seeding efficiency	
	7-day CFUs	12-day CFUs
Normal	0.15 ± 0.02[a]	0.10 ± 0.02
Treated with *M. arthritidis*	0.06 ± 0.01	0.10 ± 0.01

[a] Values are means ± SE of 2 replicate experiments.

late colonies. In this way indirect evidence was obtained for the presence of *M. arthritidis* antigens upon the surface of 7-day CFUs. To this could be attributed loss of spleen-seeding efficiency ('f' fraction) of 7-day CFUs of *M. arthritidis*-treated mice: if, as seems likely, membrane-associated mycoplasmas are expressed upon the surface of transplanted CFUs, the homing pattern of the latter might be affected.

The data presented here provides additional evidence which supports the hypothesis that the observed ability of *M. arthritidis* to activate viral murine leukemogenesis[1,5,11] in mice may result from direct action of mycoplasma organisms upon the hematopoietic target cells, causing stimulation and an increase in their numbers and/or sensitivity to R-MuLV.

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Effects of puromycin on rat embryos in vitro

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Summary. Somite-staged rat embryos were exposed to varying concentrations of puromycin for 48 h in vitro. Medium concentrations below 0.92 μM had no significant effects, while concentrations above 1.84 μM were lethal. Between these extremes, there were concentration dependent increases in the incidence of malformations in a close relationship to growth retardation.
Key words. Rat embryo; in vitro; puromycin; microbial toxin; teratogenicity; lipids.

A number of microbial toxins have been shown to be animal teratogens[1]. The ubiquitous occurrence of toxigenic microbes in the environment makes these compounds potential human teratogens. Many microbial toxins are potent inhibitors of protein synthesis[2]. Puromycin, an antibiotic produced by the soil actinomycete *Streptomyces alboniger*, is a well-characterized inhibitor of protein synthesis which acts by disrupting RNA translation[3]. Much of the data on the teratogenicity of puromycin is conflicting. It has been suggested that puromycin and other similar protein synthesis inhibitors produce embryolethality but few or no overt terata[4]. One explanation put forth for these findings is mediation of the toxic effects of puromycin through interference with placental function rather than direct effects on the embryo. The whole rodent embryo culture system developed by New and his co-workers (for review, see New[5]) has found considerable use in experimental teratology, since it allows one both to isolate the embryo from maternal effects and to examine possible mechanisms of teratogenesis. One proposed mechanism of teratogenesis is alteration of cell membrane structure or function[6]. Changes in phospholipids and cholesterol, which are important structural lipid components of cell membranes, have been associated with normal embryonic differentiation and development[7]. The present studies examine the direct effects of puromycin, a model microbial toxin, on the development of rat embryos in

Effect of puromycin on organogenesis-staged rat embryos in vitro[a]

	Puromycin (μM)				
	0	0.46	0.92	1.38	1.84
Viable/total embryos	14/14	15/16	16/16	14/18	14/20*
Impaired yolk sac/total viable embryos	0/14	0/15	0/16	5/14*	10/14*
Crown-rump length (mm)	3.26 ± 0.05 (n = 14)	3.14 ± 0.05 (n = 15)	2.87 ± 0.09* (n = 16)	2.54 ± 0.06* (n = 14)	2.11 ± 0.10* (n = 14)
Head length (mm)	1.67 ± 0.03 (n = 14)	1.65 ± 0.03 (n = 15)	1.44 ± 0.05* (n = 16)	1.31 ± 0.04* (n = 14)	0.98 ± 0.06* (n = 14)
Somites	23.4 ± 0.2 (n = 14)	23.3 ± 0.2 (n = 15)	22.2 ± 0.5 (n = 14)[b]	20.3 ± 0.7* 1(n = 7)[b]	20.7 ± 1.5* (n = 3)[b]
Protein (μg/embryo)	146.3 ± 9.1 (n = 12)	140.9 ± 9.6 (n = 13)	111.4 ± 10.3* (n = 14)	78.3 ± 5.5* (n = 12)	50.3 ± 6.6* (n = 13)
DNA (μg/embryo)	17.2 ± 1.7 (n = 12)	17.0 ± 1.3 (n = 13)	13.5 ± 1.3* (n = 14)	9.1 ± 0.9* (n = 12)	4.9 ± 0.9* (n = 13)
Phospholipid (nmol/embryo)	26.1 ± 1.8 (n = 7)	22.2 ± 2.3 (n = 7)	22.2 ± 2.6 (n = 12)	16.8 ± 1.6* (n = 12)	12.7 ± 1.8* (n = 11)
Cholesterol (nmol/embryo)	24.4 ± 1.7 (n = 7)	23.4 ± 1.3 (n = 7)	21.5 ± 0.6 (n = 12)	21.9 ± 1.8 (n = 12)	19.3 ± 1.5 (n = 11)
Abnormal/total embryos	1/14	3/15	10/16*	13/14*	13/14*

[a] Embryos were grown over gestation days 9–11 in 4 ml medium containing puromycin at the indicated concentration. Values are means ± SE. [b] Somites could not be accurately counted in some embryos in these groups. * Significantly different from control ($p < 0.05$).