MITOGENIC ACTION OF *Mycoplasma arthritidis* ON RAT LYMPHOID CELLS

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Changes in proliferative activity of lymphoid cells of rats at different times after injection with *Mycoplasma arthritidis*, were described previously [3]. Lymphocytes of infected animals 1 week after infection were found to respond to nonspecific mitogens — phytohemagglutinin (PHA) and concanavalin A (conA) — much less strongly than lymphocytes of control rats. Depression of the response to mitogens is accompanied by increased spontaneous proliferative activity of the lymphoid cells and the development of suppurative polyarthritis [1, 3]. One possible cause of these changes, it has been suggested, may be a direct mitogenic action of *M. arthritidis* on rat lymphocytes. The aim of the present investigation was to test this hypothesis.

EXPERIMENTAL METHOD

Male Wistar or Fisher rats, weighing 140-200 g, were used. The Fisher rats, which were kept under sterile conditions, were generously supplied by O. V. Chakhava, Director of the Laboratory of Gnotobiology, N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR. The mycoplasma was grown as described previously [2], and the culture poured in a volume of 1 ml into ampuls and kept at −70°C. The blast transformation reaction (BTR) was set up as described previously [3]. Besides PHA and conA, other

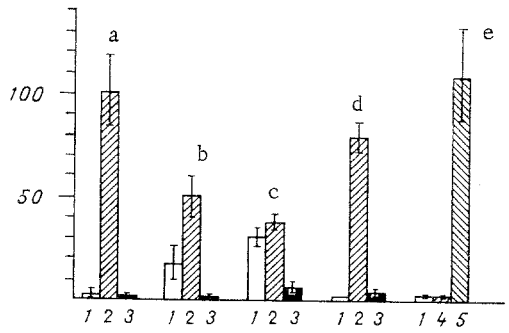

Fig. 1. Action of *M. arthritidis* on proliferative activity of rat splenocytes. Ordinate, in preparation of [^{3}H]thymidine (cpm·10^{-3}). a) Cultures without addition of mitogens, b-e) cultures stimulated by PHA, conA, LPS, and DS, respectively. 1) Control cultures inoculated with mycoplasma growth medium; 2, 3) cultures inoculated with mycoplasmas in concentrations of 10 and 40 µl per well (about 1×10^7 to 4×10^7 CFU/ml), respectively; 4) mycoplasmas in a concentration of 10 µl per well were added to the culture 1 h after DS; 5) mycoplasmas in a concentration of 10 µl per well were added to the culture 1 h before DA. Thin vertical lines indicate confidence interval at 95% probability level.

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TABLE 1. Action of Various Preparations of *M. arthritidis* on Proliferative Activity of Wistar Rat Splenocytes in Culture *in Vitro*

Agent added to cultures	Incorporation of [^{3}H]thymidine, cpm	
	experiment I	experiment II
—	—	2 045±59
Broth	1 382±698	2 599±387
M. arthritidis (10 µl per well):		
living	76 417±9 042	70 566±8 256
heated		
56°, 30 min	13 967±2 945	—
70°, 30 min	—	12 670±3 394
Filtrate:		
0,22 µ	56 087±12 016	70 894±12 032
0,1 µ	42 597±2 737	—

mitogens were used, namely dextran sulfate (DS, from Pharmacia Fine Chemicals, Sweden) in a dose of 50 µg/ml, and lipopolysaccharide from *Escherichia coli* (LPS, from Difco, USA) in a dose of 100 µg/ml. *M. arthritidis* was added to the cell cultures in a concentration of 10-40 µl/ml (1×10^7 to 4×10^7 CFU/ml). Instead of *M. arthritidis* an equal volume of mycoplasma growth medium (broth) was added to the control cultures.

EXPERIMENTAL RESULTS

Addition of *M. arthritidis* in a concentration of 10 µl per well to the lymphocyte culture led to marked stimulation of DNA replication (Fig. 1). Stimulation was comparable in value, or even higher than stimulation induced by nonspecific mitogens (PHA, conA).

After combined addition of *M. arthritidis* in a concentration of 10 µl per well and of mitogens the resultant response in the experiments with PHA was intermediate in position between the levels of proliferation observed after separate addition of mycoplasmas and PHA, but the response to conA was virtually unchanged after addition of the mycoplasmas.

Rat lymphocytes respond poorly to B-cell mitogens (DS, LPS). Under optimal conditions the index of stimulation did not exceed 3. After addition of both LPO and *M. arthritidis* (10 µl per well), the level of response was about the same as after addition of mycoplasmas alone. Conversely DS, in optimal (but not suboptimal) concentration, depressed the stimulation of DNA synthesis caused by mycoplasmas. The suppression was particularly marked if DS was added before or at the same time as the mycoplasmas.

The stimulating action of *M. arthritidis* was not exhibited if its concentration in the culture was increased fourfold (to 40 µl per well). In that case the mycoplasmas blocked the response of the lymphocytes to all mitogens studied practically completely.

Stimulation of DNA synthesis in the presence of mycoplasmas may be due to at least three different causes: 1) proliferation of microorganisms in culture; 2) the specific response of lymphocytes to mycoplasmal antigen (if the animals were contaminated beforehand with *M. arthritidis*); 3) the mitogenic action of the mycoplasmas.

Proliferation of the microorganisms could not have been the main cause of stimulation for the following reasons: 1) They were cultured in the presence of 100 µg/ml of streptomycin, which inhibits growth of *M. arthritidis*; 2) during precipitation of the label the cells were washed on filters with a pore diameter of 0.4 µ, which allow mycoplasmas to pass through freely; 3) an increase in the concentration of *M. arthritidis* would have led to increased incorporation of [^{3}H]thymidine, whereas the opposite effect was observed; 4) the majority of cells in culture consisted morphologically of blast cells; mycoplasmal growth medium, freed from microorganims by filtration, likewise possessed stimulating properties (Table 1). It is more difficult to choose between the last two possible reasons. The fact that maximal stimulation under the influence of *M. arthritidis* was observed on the 3rd day and was comparable in strength with the action of PHA and conA is difficult to explain purely by the specific response of sensitized lymphocytes. Moreover, experiments on germ-free Fisher rats (Table 2) gave the same result as experiments on rats kept under ordinary conditions.

TABLE 2. Action of *M. arthritidis* on Proliferative Activity of Rat Splenocytes

Animals	Agent added to cultures	Incorporation of ^{3}H-thymidine, cpm
Ordinary	—	266±78
	Broth	533±285 (2,0)
	M. arthritidis (10 μl per well)	7 064±2 851 (26,6)
Germfree	—	760±115
	Broth	1 502±376 (1,9)
	M. arthritidis (10 μl per well)	13 501±6 419 (17,7)

Legend: stimulation index given in parentheses.

The facts are thus evidence that *M. arthritidis*, in a dose of about 10^7 CFU/ml, has a mitogenic action on lymphocytes, probably associated with a factor produced by the mycoplasma. A similar mitogenic action of *M. arthritidis* on rat lymphoyctes has been observed by other workers [7], although they did not undertake a detailed study of this effect. It was shown [4, 5] that in doses of under 10^6 CFU/ml, *M. arthritidis* stimulates mouse lymphocytes, but in higher concentrations it inhibits the BTR induced by mitogens. Heating the mycoplasmas for 30 min at 56°C caused the inhibitory effect to be replaced by stimulation. Since inhibition of the BTR by mycoplasmas is known to be due to the removal of arginine from the culture medium [8], the absence of inhibitory properties in a heated culture of mycoplasmas indicates that arginine deiminase is more thermolabile than the mitogenic factor. In our experiments (Table 1) heating never led to the change from inhibition to stimulation, but the mitogenic effect was inhibited by heating (although not even keeping the culture at 70°C for 30 min completely abolished the BTR induced by *M. arthritidis*). Possibly *M. arthritidis* produces several mitogenic factors, differing in thermolability.

It is not yet clear which cells undergo blast transformation under the influence of *M. arthritidis*. Cole et al. [5] showed that treating mouse lymphocytes with anti-Q-serum abolishes the reaction. Of all the mitogens tested in the present experiments only DS was able to depress the mitogenic action of *M. arthritidis*; the effect, moreover, was stronger in the case when DS was added before, but not after, the mycoplasmas. One possible explanation of these findings is that DS and mycoplasmas react with the same lymphocyte subpopulation. Meanwhile we know that DS stimulates immature B cells in mice [6]. The possibility therefore cannot be ruled out that B cells also take part in the BTR under the influence of mitogenic factor of *M. arthritidis*.

The action of *M. arthritidis* on rat lymphocytes thus depends essentially on the dose of the microorganisms. In high concentrations a culture of this mycoplasma inhibits BTR of lymphocytes, but in concentrations below 10^7 CFU/ml it has a mitogenic action.

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