ROLE OF THYMUS-DEPENDENT CELLS IN SPLENIC COLONY FORMATION

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Treatment of bone marrow with serum reacting with theta-antigen, irrespective of the presence of complement, sharply reduces the ability of its cells to form splenic colonies. Injection of thymus cells into the recipient together with bone marrow cells considerably reduces the effect of this serum and substantially increases splenic colony formation. It is suggested that the antiserum inactivates a cell population in the bone marrow that is essential for colony formation in the spleen, but which differs from the pluripotent stem cells—probably the T cell population

KEY WORDS: pluripotent stem cells; splenic colony formation; serum against theta-antigen; T cells.

It was shown previously [2] that when rabbits are immunized with mouse brain, antibodies cross-reacting with the theta-antigen of thymus cells are formed in them. It was later shown that such sera are active against pluripotent stem cells capable of forming splenic colonies. It has also been reported that differences between resting pluripotent and committed stem cells [6], and stem cells which have passed through different numbers of generation cycles [4], can be detected by means of antibrain sera.

The writers showed previously that treatment of bone marrow with concanavalin A, a substance causing blast-transformation of thymus cells, leads to a significant decrease in the number of colony-forming cells in it. This effect is almost totally abolished by injection of intact thymus cells. On the basis of these results it was postulated that cooperation between stem cells and T cells is essential for splenic colony formation [1].

The action of sera cross-reacting with theta-antigen on colony formation was investigated in the experiments described below.

EXPERIMENTAL METHOD

Experiments were carried out on male (CBA × C57BL)F_1 mice about 3 months old. Colony-forming activity of bone marrow suspensions was studied by the splenic exocolonies method [5]. Rabbit immune serum against mouse theta-antigen was obtained by the method described previously [2], and the working dilution was 1:3. A suspension of bone marrow cells containing $2 \cdot 10^7$ cells/ml was incubated with serum against mouse theta-antigen in the following proportions by volume: 0.1 ml suspension, 0.1 ml serum, 0.1 ml complement (or Hanks' solution), for 1 h at 37°C. After the end of incubation the samples were washed with cold Hanks' solution by centrifugation at 3000 rpm for 10 min. After washing, the cells were resuspended in 10 ml Hanks' solution and injected intravenously into lethally irradiated recipients (850 rad, ^{60}Co γ-rays). Instead of bone marrow cells, some groups of recipients received injections of $1.75 \cdot 10^7$ syngeneic thymus cells. The mice were killed 8 days later, the spleens were fixed, and the number of splenic colonies was counted.

EXPERIMENTAL RESULTS

As Table 1 shows, treatment of bone marrow with serum reacting with theta-antigen, whether or not complement was present, sharply reduced the ability of its cells to form splenic colonies. These results are in good agreement with data in the literature. They were interpreted previously as the result of the direct action of antisera containing antibodies against theta-antigen on pluripotent stem cells. It was found, however, that if thymus cells were injected together with bone marrow cells into the recipient, the effect of these sera was largely abolished and splenic colony formation increased significantly. These investigations showed that

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TABLE 1. Effect of Incubation of Bone Marrow in vitro with Serum against Mouse Theta-Antigen on Exogenous Splenic Colony Formation

Group	Group		Number of mice	Number of colonies per spleen (M ± m)	P
1	Control	Incubation for 1h at 37°C	29	12,9±0,9	
2	Control + thymus cells		24	15,7±1,2	
3	Serum against theta-antigen		32	1,9±0,4	<0,001
4	Serum against theta-antigen + thymus cells		15	7,0±1,0	
5	Serum against theta-antigen + complement		19	1,9±0,5	<0,001
6	Serum against theta-antigen + complement + thymus cells		18	9,0±1,1	
7	Complement		20	11,9±0,9	
8	Unincubated control		12	13,0±0,8	
9	Unincubated control + thymus cells		12	12,2±0,8	
10	Endogenous background		17	0,3	
11	Endogenous background + thymus cells		14	0,7	

Note. Mean number of colonies given per $1 \cdot 10^5$ bone marrow cells injected.

the effect of thymus cells can only be explained on the grounds that a cell population playing an important role in splenic colony formation, but different from the pluripotent stem cells, was inactivated by the antiserum in the bone marrow. These cells contain theta-antigen and can be replaced by thymus cells, i.e., they were evidently T cells. Another interesting fact is that during incubation some of the T cells essential for colony formation probably lose their activity, or the addition of intact thymus cells somewhat increased the yield of splenic exocolonies (Table 1, groups No. 1 and 2).

The results described above, together with those obtained previously concerning the effect of concanavalin A on splenic colony formation, thus suggest that thymus cells play an important role in splenic colony formation. Probably direct contact (cooperation) between the pluripotent stem cell and the thymus cell is essential. In this way the marked inhibition of colony formation by the antisera in the absence of complement could be explained. The same phenomenon has been observed by other workers also [6]. The results of the present experiments, together with an analysis of data in the literature [3], suggests that cooperation with T cells is essential for pluripotent but less important for committed stem cells, growing in agar. Antiserum against theta-antigen has no action on the population of committed cells forming granulocytic colonies in agar [6].

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