Formation of Colonies of Clonogenic Stromal Precursors in Bone Marrow and Splenic Cell Cultures in the Presence of Streptococcal Antigens in the Culture Medium

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The presence of streptococcal M protein and A polysaccharide in culture medium is shown to have an inhibitory effect on the growth of clonogenic stromal precursors in cultures of healthy murine bone marrow and of healthy guinea pig bone marrow and spleen. The efficacy of colony formation dropped 1.5- to 2-fold in the presence of antigens in a concentration of 25 μg/ml in the medium. The inhibitory effect was absent if antigens were added to adhesive cell cultures. The addition of antigens to cultures originating from animals immunized with streptococcus resulted in inhibition of the efficacy of colony formation in complete cultures and in cultures of adhesive cells. The presence of streptococcal antigens in guinea pig stromal fibroblast cultures of different strains did not affect their growth or colony formation. These data indicate that the effects of streptococcal antigens appear to be aimed at the stromal cells not directly, but rather via another cellular category in the bone marrow and splenic cell cultures, probably lymphocytes.

Key Words: *stromal cells; streptococcal antigens*

Stromal cells of hemopoietic and lymphoid organs play an important role in hemopoiesis and immunity, creating a microenvironment for the hemopoietic and immunocompetent cells. During explantation of cells of hemopoietic and lymphoid organs in monolayer cultures, clonogenic stromal precursor cells form colonies - fibroblast clones - and the number of these colonies allows the number of clonogenic stromal precursors (COC-F) in the respective cell suspensions to be estimated [5]. The number of COC-F in the lymph nodes and spleen increases appreciably after administration of certain antigens [1]. It has been found that macrophages and lymphoid cells activated by antigens and mitogens and by the factors they produce have a noticeable impact on *in vitro* COC-F proliferation [2,4].

This study is devoted to the effects of the streptococcal antigens M protein and A polysaccharide (A-PS) on the growth of stromal colonies in cultures of bone marrow cells and splenocytes from intact animals and animals immunized with group A streptococci. It should be borne in mind that bone marrow and splenocyte monolayer cultures explanted with a fairly high cell density contain, besides stromal cells, numerous immunocompetent cells capable of reacting to the presence of an antigen in the culture medium. That is why one of the problems investigated here was whether the effects of antigens present in the cultures are aimed directly at the stromal cells or are medi-

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ated by other categories of cells associated with COC-F *in vitro*.

MATERIALS AND METHODS

Experiments were carried out with CBA mice and guinea pigs, intact or immunized with type 5 streptococcus culture killed by heating at 56°C for 1.5 h. Streptococcal cultures were injected intraperitoneally: to guinea pigs in a single dose of 22×10^9 bacterial cells, to mice once a week for 4 weeks in a dose of 2×10^9 bacterial cells. Bone marrow and spleen were removed 3 weeks after immunization. Cell suspensions were prepared as described previously [5]. Bone marrow cells (10^6) and splenocytes (4×10^6 per well) were explanted in 12-well plates in α-MEM medium (Flow) with 15% fetal calf serum.

Cultures of two types were used: common complete cultures of bone marrow cells and splenocytes containing all cells present in these organs and cultures of adhesive cells of the bone marrow free of bone marrow cells nonadhesive to plastic. In order to prepare adhesive cultures, bone marrow cells and splenocytes were incubated for 2 h at 37°C, after which nonadhesive cells were discarded, and subsequent culturing was carried out in the same medium, but with the addition of a feeder, bone marrow cells of a guinea pig irradiated in a dose of 60 Gy [3].

For the preparation of stromal fibroblast cells, 3×10^7 bone marrow cells and 6×10^7 splenocytes of intact and immunized guinea pigs were explanted in flasks with a bottom area of 50 cm². After 7 to 10 days the cells were removed with 0.25% trypsin, resuspended in α-MEM medium, and transferred to a new flask. Cells were cultured in α-MEM medium with 15% fetal calf serum. The second or third passages of fibroblasts of such strains were explanted in 12-well plates, 200-400 per well. Streptococcal M protein and A-PS prepared as described previously [6,7] were added to the wells in concentrations of 25 and 5 μg/ml 1 h after cell explantation. The preparations were kindly supplied by V. Yu. Sanina and N. A. Borodiyuk (N. F. Gamaleya Research Institute of Epidemiology and Microbiology). The cultures were fixed after one week, and then either the number of fibroblast colonies containing at least 50 cells (in primary cultures of bone marrow cells and splenocytes), or the total number of fibroblasts (in cultures of passaged strains) was counted. In the former case the efficacy of colony formation (ECF-F) was assessed, that is, the number of COC-F colonies per 10^4 (in mice) or 10^5 (in guinea pigs) cells of explanted bone marrow or per 10^5 splenic cells was estimated.

TABLE 1. Efficacy of Cloning of Stromal Clonogenic Cells in Mouse Bone Marrow Cultures in the Presence of Streptococcal Antigens ($M\pm m$)

Antigen in medium, μg/ml		ECF-F, $\times10^{-4}$	
		complete cultures, 10^6 cells explanted	adhesive cultures, 10^5 cells explanted + 2.5×10^6 feeder cells
Intact			
M protein	–	0.42±0.04	1.6±0.2
	25	0.18±0.04	1.3±0.1
	5	0.26±0.03	1.5±0.1
A–PS	–	0.34±0.01	1.5±0.1
	25	0.20±0.01	1.4±0.2
	5	0.23±0.01	1.2±0.1
Immunized with streptococcus			
M protein	–	0.32±0.04	1.2±0.1
	25	0.23±0.07	0.7±0.2
	5	0.29±0.04	0.7±0.2
A–PS	–	0.38±0.01	1.5±0.1
	25	0.28±0.04	0.9±0.1
	5	0.33±0.05	1.5±0.4

RESULTS

The efficacy of cloning of stromal clonogenic cells in complete cultures of intact murine bone marrow after explantation of 10^6 cells was $0.42\pm0.04\times10^{-4}$. The addition of M protein to the culture suppressed ECF-F 2.3-fold at an antigen concentration in the medium of 25 μg/ml. In a concentration of 5 μg/ml the antigen had a less pronounced effect on ECF-F value (Table 1).

Similar results were observed after the addition of streptococcal A-PS to the culture medium: the efficacy of cloning dropped at A-PS concentrations of

TABLE 2. Growth of Stromal Cells in Primarily Explanted Cultures of Guinea Pig Bone Marrow and Spleen from Intact Animals and Those Immunized with Streptococcus in the Presence of M Protein ($M\pm m$)

Animals	Antigen in medium, μg/ml	ECF-F, $\times10^{-5}$	
		bone marrow	spleen
Intact	–	1.7±0.2	1.9±0.1
	25	1.8±0.4	0.8±0.1
Immunized	–	2.7±0.2	1.5±0.1
	25	1.9±0.2	0.7±0.1

TABLE 3. Growth of Stromal Cells in Guinea Pig Bone Marrow and Splenic Strains Passaged Several Times in the Presence of Streptococcal M Protein ($M \pm m$)

Cells	Animals	Antigen in medium, μg/ml	Strains after passages	
			ECF-F, $\times 10^{-2}$	number of cells per well, $\times 10^3$
Bone marrow	Intact	–	14±1	5.9±0.4
		25	18±1	6.6±0.4
	Immunized	–	12±2	4.5±0.5
		25	12±1	6.0±0.2
Spleen	Intact	–	24±2	10.1±1.1
		25	24±2	9.2±1.4
	Immunized	–	48±3	36.5±0.4
		25	48±3	34.0±2.1

25 and 5 μg/ml. The addition of M protein and A-PS to cultures of adhesive bone marrow cells of normal mice did not suppress COC-F growth (Table 1).

The presence of streptococcal antigens in intact guinea pig bone marrow cultures had virtually no effect on ECF-F. For example, in experiments with M protein ECF-F ($\times 10^{-5}$) was 1.7±0.2 without antigen and 1.8±0.4 in the presence of antigen (Table 2).

The next series of experiments was carried out with bone marrow and splenocyte cultures of animals immunized with streptococcal culture. The addition of M protein and A-PS to murine bone marrow cultures led to inhibition of ECF-F both in complete and in adhesive cell cultures (Table 1). The same effect was observed in complete bone marrow cell cultures derived from immunized guinea pigs (Table 2).

A study of the effect of streptococcal antigen on guinea pig splenocyte cultures originating from intact and immunized animals showed that in the presence of M protein the ECF-F value in both cultures dropped approximately twofold (Table 2).

In order to find out whether streptococcal antigens may directly influence the stromal cells or whether their action is mediated by other categories of cells present in the cultures, strains of stromal bone marrow and splenic fibroblasts of intact guinea pigs and guinea pigs immunized with group A streptococcus were prepared. These strains are known to contain only stromal cells and to be free of cells of other categories. The presence of streptococcal antigens in different strains of stromal fibroblast cultures was found to have no impact on their growth *in vitro,* no matter what the source of the strain was, intact animals or animals immunized with streptococcus (Table 3).

The most important result gleaned from this study is that we found appreciable differences in the ECF-F value under the effect of streptococcal antigens in adhesive bone marrow cultures from intact and immunized animals. The growth of stromal colonies in complete bone marrow cultures from intact and immunized mice was inhibited in the presence of these antigens, whereas in normal murine adhesive cultures the inhibitory effect was absent and, vice versa, was clearly seen in adhesive cultures from immunized animals. These data indicate that the stromal cells do not by themselves react to streptococcal antigens in a culture and that the inhibitory effect may be due to the presence of lymphoid cells recognizing the antigens in complete cultures. A few lymphocytes appear to be present in adhesive cell cultures from immune animals.

These data are in line with the results of studies with bone marrow and splenic fibroblasts of intact and immunized guinea pigs. The addition of M protein did not inhibit cell proliferation in any of these strains, whereas a manifest inhibitory effect of COC-F colony formation was observed in complete primary cultures of guinea pig splenocytes.

On the whole, we get the impression that the inhibitory effect of the antigens on colony formation is related to the presence of lymphocytes in the cultures which immunologically recognize these antigens and evidently thus release the factors influencing the proliferation of stromal fibroblasts. The degree of inhibition caused by different antigens apparently varies. Our experiments showed a much less pronounced inhibition of ECF-F than that caused by tuberculin added to cultures of guinea pig splenocytes and bone marrow cells [4].

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