HETEROLOGOUS ANTISERA AGAINST STROMAL MECHANOCYTES OF BONE MARROW

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Heterologous rabbit antifibroblast serum (AFS) against stromal fibroblasts of guinea pig bone marrow was obtained. Both in the cytological reaction for adherent cells and in the indirect immunofluorescence test AFS specifically bound fibroblasts and their precursor cells (but not macrophages) in monolayer primary cultures of bone marrow, thymus, and spleen cells, and also precursor cells of fibroblasts of the blood and peritoneal exudate of guinea pigs (in the cytotoxic reaction). Fibroblast precursor cells of the thymus were more sensitive to the action of AFS than splenic and bone marrow cells, which suggests a higher concentration of common tissue-specific antigens on the stromal mechanocytes of the thymus. Precursor cells of stromal fibroblasts in native cell suspensions were essentially more sensitive to the cytotoxic action of AFS than colony-forming fibroblasts from passage cultures.

KEY WORDS: antifibroblast serum; stromal mechanocytes.

Antisera binding with fibroblasts but not with other cells can be obtained by immunization with whole fibroblasts [5], collagen and procollagen, and also with preparations of the basic surface protein of fibroblasts [7, 9, 10].

The object of the present investigation was to obtain an antiserum against stromal fibroblasts of bone marrow which are responsible for the transfer of the hematopoietic microenvironment [4].

EXPERIMENTAL METHOD

Rabbits were immunized with pure cultures of fibroblasts (stromal mechanocytes) of guinea pig bone marrow after 3 to 10 passages [4]. About $2 \cdot 10^7$ fibroblasts, removed with 0.25% trypsin solution (treatment for 30 min at 37°C) and washed 5 times with Hanks' solution were used for each immunization. The first immunization was carried out with Freund's adjuvant into the heel. The three subsequent immunizing injections were given intravenously after intervals of 2 weeks and 1.5 and 2 months. Ten days after the last immunization a serum was obtained, inactivated at 56°C for 30 min, and kept at −70°C. Serum taken before the beginning of immunization was used as the control.

The antifibroblast serum (AFS) was exhausted with guinea pig red blood cells (RBC) (5 volumes AFS+1 volume RBC, 1 h at 4°C), with normal bovine serum (NBS) (5 volumes AFS + 1 volume NBS, 45 min at room temperature), with guinea pig bone marrow, spleen, peritoneal exudate, and thymus cells (1 volume AFS+1 volume of cells, 1 h at room temperature, then overnight at 4°C), with guinea pig liver powder (about 50 mg/ml, 1 h at room temperature), and with normal guinea pig serum (1 volume AFS+1 volume normal serum, 30 min at room temperature). After exhaustion the AFS was used in the indirect immunofluorescence test and the cytotoxic reaction.

The action of AFS was tested on primary monolayer cultures of guinea pig bone marrow, spleen, thymus, peritoneal exudate, and blood cells and also on passage cultures of stromal mechanocytes of the bone marrow, thymus, and spleen [2-4].

Antiserum against rabbit γ-globulins labeled with isothiocyanate (Sevac, Czechoslovakia) mixed 1:1 with rhodamine-labeled albumin was used for the indirect immunofluorescence test with AFS. The specificity of

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TABLE 1. Cytotoxic Action of AFS on Precursor Cells of Stromal Mechanocytes (primary cultures)

Dilution of AFS*	Percentage inhibition of colony formation†		
	thymus	spleen n	bone marrow
1:10—1:32	97,8±1,25	91,5±1,55	87,0±5,14
1:40—1:80	91,7±4,66	86,0±2,85	84,4±7,31
1:100—1:160	96,0±1,73	90,0±0,50	92,4±1,76
1:256—1:320		83,0±0,50	92,5±2,50
1:512—1:640	—	95,0±0,50	94,0±2,00
1:1000—1:1300	97,4±2,18	90,0±5,00	72,5±8,50
1:5000	85,5±10,5	87,0±1,00	61,5±11,5
1:10 000	89,5±2,69	—	0
1:100 000	50,0±5,00	0	0

*Cultures treated with AFS (exhausted by guinea pig RBC and NBS) and with complement 6-18 h after adhesion of cells to flasks. After treatment for 1.5 h (20 min with AFS and 1 h with AFS and complement at 37°C) and washing of cultures with medium No. 199 flasks were filled with complete culture medium and cultured in the presence of 5% CO_2 for 10-14 days, after which number of discrete fibroblast colonies was counted.
†Number of colonies growing after treatment of cultures with normal rabbit serum was taken as zero (i.e., absence of suppression).

TABLE 2. Cytotoxic Action of AFS on Precursor Cells of Stromal Mechanocytes (passage cultures)

Dilution of AFS*	Percentage inhibition of colony formation†		
	thymus	spleen	bone marrow
1:100	100	95,25±2,85	100
1:100	100	76,10±14,1	97,85±2,15
1:1000	0,00	0,00	0,00

*Conditions of treatment of stromal mechanocytes adherent to glass the same as for primary cell cultures (see the section "Experimental Method").
†Number of colonies growing when normal rabbit sera were used in a dilution of 1:10 was taken as the zero level (absence of suppression).

the test was verified by means of controls [1]. The test was carried out by the method described in [6]. The labeled antiserum was exhausted with mouse liver powder and with cells that served as the source for the cultures.

The cytotoxic reaction was carried out in suspension and the results were assessed by the trypan blue elimination method [8] and in cells adherent to glass. For this purpose, $1 \cdot 10^6$-$40 \cdot 10^6$ cells of the hematopoietic and lymphoid organs or $5 \cdot 10^2$-$2 \cdot 10^3$ passage stromal mechanocytes were explained in 100-ml flasks. The cultures were washed 6-18 h later with medium No. 199 without serum and treated with 0.5 ml AFS of the corresponding dilution for 20 min. After 60 min 1 ml of guinea pig complement was added to each flask and the contents were incubated at 37°C, after which the serum and complement were replaced (after washing) by complete culture medium with the addition of $1.5 \cdot 10^7$-$2 \cdot 10^7$ bone marrow cells irradiated in a dose of 4000 R (as feeder). The number of fibroblast colonies growing in the flasks was counted after 10-14 days. Cultures treated with normal rabbit serum and complement served as the control. The cytotoxic reaction also was set up on cover slips (without the addition of the feeder). The number of macrophages and fibroblasts in these cultures was counted on the third day.

EXPERIMENTAL RESULTS

In the cytotoxic test in the presence of complement, AFS exhausted by RBC and NBS in a dilution of 1:16 gave cytotoxic indices of zero for thymus and bone marrow cells in suspension. The same AFS, acting on guinea pig bone marrow cells after adhesion to the glass, did not lead to a decrease in the number of growing macrophages, but killed about 90% of the fibroblasts. Data on the action of AFS on the formation of fibroblast colonies in primary cultures of thymus, spleen, and bone marrow cells are given in Table 1. During the action of AFS on guinea pig peritoneal exudate and blood cells, inhibition of colony formation was 97% and 86%, respectively. Cytotoxic action of ASF on colony formation in the passage cultures of stromal fibroblasts is shown by the data in Table 2. Exhaustion of AFS with normal guinea pig serum completely suppressed the cytotoxic reaction.

In the immunofluorescence test the AFS used were exhausted by two methods: 1) with RBC only (in a dilution of 1:16-1:64); 2) with RBC, NBS, peritoneal exudate (cells), and liver powder (in a dilution of 1:4). In both cases all fibroblasts in 9-15-day cultures of guinea pig bone marrow, thymus, exudate, and spleen cells specifically bound AFS. Histiocytes in the same cultures did not react with AFS. Exhaustion of AFS by normal guinea pig serum completely suppressed the reaction.

These results show that heterologous AFS obtained by immunization with pure cultures of bone marrow fibroblasts contain specific antibodies against stromal mechanocytes both of bone marrow and of other organs – thymus and spleen. These antibodies are found in the complement-dependent cytotoxic reaction and in the immunofluorescence test with exhausted serum, which does not act in the same reactions on other cells (hematopoietic cells, lymphocytes, and macrophages).

The results show that tissue-specific antigens of fibroblasts are also present on cells forming clone-colonies in cultures, and also on precursors of fibroblasts present in circulating blood and peritoneal exudate of guinea pigs.

Treatment with AFS, although not affecting macrophage formation in cultures, inhibits growth of fibroblast colonies. This confirms that macrophages and mechanocytes in fact have different precursor cells [1] and that mechanocytes are not formed from hematopoietic and lymphoid cells. AFS is a convenient means of distinguishing fibroblasts from macrophages in cases when this is difficult morphologically and also for purifying cell populations from fibroblast precursors.

The AFS used in this investigation was obtained against bone marrow fibroblasts. In the indirect immunofluorescence test it was impossible to detect any difference in its activity relative to fibroblasts of different origin. Meanwhile, the cytotoxic reaction of inhibition of fibroblast colony formation showed that colony-forming precursors of fibroblasts of thymus origin are more sensitive to the action of AFS than those of splenic and bone marrow origin: The dilutions of AFS inhibiting colony formation in primary cultures of the thymus did not inhibit it in primary cultures of bone marrow and spleen cells. These differences in sensitivity were less marked in passage cultures of fibroblasts.

Colony-forming presursor cells of fibroblasts in native cell suspensions were in general significantly more sensitive to the cytotoxic action of AFS than colony-forming fibroblasts from passage cultures. These observations could indicate a difference in the concentration of common tissue-specific antigens on fibroblast presursor cells obtained from different sources.

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