Counts of Stromal Precursor Cells in Heterotopic Bone Marrow Transplants in Mice Immunized with Group A Streptococcus Antigens

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The count of stromal precursor cells in bone marrow transplants from CBA mice, transplanted to animals immunized with killed type 5 group A streptococcus vaccine, decreased 4.5-6.5 times (depending on the transplant age) in comparison with the grafts transplanted to normal recipients. The counts of stromal precursor cells in 1.5-3-month bone marrow transplants from animals immunized with killed streptococcal vaccine transplanted to normal mice were virtually the same, while in 7-month transplants they decreased 2-fold in comparison with their counts in bone marrow transplants from normal CBA mice transplanted to normal animals. The content of stromal precursor cells in the femoral bone marrow of animals immunized with killed streptococcal vaccine was appreciably (3.5 times) higher than in the bone marrow of normal mice. The results attest to an appreciable effect of streptococcal antigens on the bone marrow stromal tissue and suggest that not all stromal precursor cells, whose count increases after injection of antigens, are responsible for transplantability of the stromal tissue in case of its heterotopic transplantation.

Key Words: *bone marrow stromal cells; immune response; streptococcal antigens*

Bone marrow stromal tissue contains precursor cells of many cell lines and osteogenic precursor cells [4, 11]. After transplantation of bone marrow fragments under the renal capsule, a bone marrow organ populated by hemopoietic cells develops from stromal stem precursor cells responsible for transplantability within 2-3 weeks at the site of bone marrow fragment transplantation [4]. Bone tissue is continuously restructuring during the entire life span of the individual. Live organism permanently face infections, and therefore reorganization of part of bone tissue by stromal stem cells can be realized in the presence of an infectious process. A questions arise, how infectious processes often followed by autoimmune states can modify stromal tissue and what are the consequences (immediate and delayed) of these situations. We tried to answer these questions using a model of heterotopic bone marrow transplantation to animals immunized with streptococcal antigens, the most prevalent infection often provoking the development of autoimmunity. Group A streptococcus infection can lead to the development of so-called poststreptococcal diseases (rheumatic fever and glomerulonephritis) associated with autoimmunity. These patients have autoantibodies, autoreactive T-cells, and bound immunoglobulins in the target organ [5,6]. According to modern concepts, the main cause of autoimmune reaction in these diseases are cross-reacting group A streptococcus antigens, identical to tissue antigens of heart, kidney, skin, thymus, and other human and animal organs [1,5]. After immunization of animals (rabbits,

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mice) with cultures of some streptococcus types (1, 5, 12, *et al.*), containing cross-reacting antigens, autoantibodies reacting with normal tissue components were formed in the sera of these animals [1,5]. Hybrydomas producing monoclonal antibodies cross-reacting with human and animal tissue antigens were obtained by immunization of mice by group A streptococcal cultures [3]. Another aspect in this problem is the use of stromal tissue transplantation for the treatment of some pathologies [8]. It was therefore interesting to clear out, whether enrichment of cell populations used for transplantation with stromal clonogenic cells (CFC-F) can be useful, in other words, to evaluate the ratio between the count of CFU-F and stem stromal cells responsible for transplantability of the stromal tissue. There are data that not all CFC-F forming colonies in cultures are responsible for stromal tissue transplantability in case of its heterotopic transplantation [4].

The objective of this study were as follows: to clear out how the number of CFC-F is changing in bone marrow transplants 1) from normal CBA mice, developing under the renal capsule of normal mice, in comparison with mice immunized with streptococcal antigens, 2) in bone marrow transplants from mice immunized with streptococcal antigens, developing under the renal capsule of normal mice, in comparison with transplants from normal mice, and 3) to evaluate the effect of immunization with streptococcal antigens on CFC-F count in the bone marrow of immunized mice.

MATERIALS AND METHODS

Experiments were carried out on 2-3-month-old CBA mice and 4-5-month-old guinea pigs from Kryukovo Central Breeding Center. The mice were immunized with killed group A streptococcus vaccine (gracious gift from Dr. N. A. Borodiyuk, Laboratory of Immunity Regulation, N. F. Gamaleya Institute). In order to prepare the vaccine, streptococci were cultured for 18 h at 37°C, bacterial cell precipitate was centrifuged for 30 min at 2,000 rpm, washed 3 times with 0.85% NaCl, and heat-inactivated in a water bath at 56-58°C for 1 h. The suspension was lyophilized and stored in a refrigerator at 4°C; 1 mg dry mass contained about 2 billions bacterial cells. The mice were intraperitoneally immunized with the vaccine in ascending doses during 3 weeks (3 consecutive days in each week): 1 billion bacterial cells/injection on week 1, 2 billions on week 3, and 3 billions on week 3. Immunization of animals (rabbits, mice) according to this protocol leads to the formation (in addition to antibodies to streptococcal antigens) of autoantibodies to various tissue antigens, the maximum antibody production was observed on days 7-14 after the last injection of the antigens [1,3]. Autoantibodies to heart tissue antigens were detected in the sera of immune mice by indirect immunofluorescence on bovine heart tissue sections. We used FITC-labeled serum to mouse immunoglobulins (N. F. Gamaleya Institute). The method for staining of sections and interpretation of the results was described previously [6]. For heterotopic transplantation half of the contents of murine femoral bone marrow cavity was injected under the renal capsule of these animals as described previously [4] in the following donor-recipient combinations: normal—normal, normal—immune (bone marrow was transplanted on day 8 after the end of immunization of recipients), immune—normal (bone marrow transplantation on day 12 after immunization of donors).

Bone marrow cell suspensions from mice and guinea pigs were prepared with a syringe, as described previously [7]. Cell suspensions of the bone marrow from the transplant were prepared as follows: the content of bone marrow capsules was scraped with a scalpel into α-MEM (Sigma) with 5% FCS (Paneco), passed several times through a syringe with needles of decreasing diameter, and filtered through 4-fold capron filters. Bone marrow cells ($5\text{-}20\times10^5$) were explanted into 25-cm^2 flasks in 5 ml culture medium (α-MEM) with 20% FCS (Paneco) with antibiotics (penicillin and streptomycin, 100 μg/ml each). Bone marrow cells (1.5×10^7) from guinea pigs exposed to 60 Gy (Co 60, 10 Gy/min) were added to all cultures as a feeder. The cultures were incubated for 12 days in a CO_2 incubator at 37°C, fixed in ethanol, stained with azur-eosin, and colonies containing at least 50 fibroblasts were counted. The efficiency of cloning (ECF-F) was evaluated by the number of colonies (number of colonies formed by 10^5 explanted cells).

RESULTS

The presence of autoantibodies in immune mouse sera was confirmed by indirect immunofluorescence on sections of bovine heart tissue. Antibodies reacting with sarcolemmal and subsarcolemmal antigens of myocardial fibers were detected. It was previously shown that these antibodies are directed to organ-specific myocardial antigens common for animals of different species (rabbit, mouse, ox, *etc.*), that is, they were autoantibodies [1,3]. Hence, immunization of animals according to the above protocol results in production of not only antibodies to streptococcal antigens, but also autoantibodies. The content of nucleated cells in the studied variants of transplants changed little in comparison with the control within the same age groups (by no more than 40%, Table 1). However, the content of nucleated cells in 7-7.5-month-old transplants in comparison with 2.5-3-month-old ones increased only after transplantation from normal donors

TABLE 1. ECF-F in Bone Marrow Transplants of Normal Mice and Mice Immunized with Group A Streptococcus Culture

Transplant age, transplantation		Number of nucleated cells per transplant, $\times 10^6$	ECF-F, $\times 10^{-5}$	Content of CFC-F per transplant
1.5-months	normal to normal	1.2±0.2	6.7±0.7	80±8
	normal to immune	0.9±0.2	1.9±0.2	17±2
	immune to normal	1.8±0.4	3.8±0.3	68±5
2.5-3 month	normal to normal	3.6±0.2	1.3±0.2	47±7
	normal to immune	3.6±0.6	0.3±0.1	11±3
	immune to normal	5.2±0.6	0.8±0.2	41±10
7-7.5-month	normal to normal	6.2±1.1	0.5±0.1	33±6
	normal to immune	3.9±0.7	0.1±0.0	5±1
	immune to normal	3.9±0.8	0.4±0.1	15±3

TABLE 2. ECF-F in Cultures of Femoral Bone Marrow from Normal Mice and Mice Immunized with Group A Streptococcus Culture

Day after immunization	Number of nucleated cells per femur, $\times 10^6$	ECF-F, $\times 10^{-5}$	CFC-F content per femur
Normal (no immunization)	9.0±0.1	1.4±0.3	123±33
8	10.0±1.1	4.8±0.6	480±61
12	12.4±1.6	3.4±0.6	427±72

to normal recipients (1.7 times; Table 1). ECF-F in bone marrow transplant cell cultures and the content of CFC-F transplanted from normal donors to immune recipients decreased 3.5 and 4.7 times in 1.5-month, 4.3 times in 3-month, 4.2 and 6.6 times in 7-month transplants in comparison with the normal-to-normal transplants. The percentage of colonies with alkaline phosphatase activity (osteogenesis marker) also decreased in bone marrow transplant cell cultures in the normal-to-immune variant (15.5±1.2%) in comparison with the normal-to-normal variant (37.8± 7.8%). ECF-F and CFC-F values in bone marrow transplants of the immune-to-normal variant also decreased in comparison with the normal-to-normal variant, but to a far lower degree: the maximum drop of ECF-F (by 40%) was observed in 1.5- and 3-month transplants, while the maximum drop of CFC-F count (2.2 times) was observed in 7-month transplants. These data seem to indicate that the developing and existing in immunized organism bone marrow organ (normal-to-immune variant) is defective by ECF-F (by concentration) and by the content of stromal precursor cells. We should remember that as soon as 3 weeks after transplantation bone marrow graft is completely formed and its bone tissue is formed even earlier, during the first 2 weeks [4]. Hence, the formation of the transplant coincides with the period of maximum antibody production (including autoantibodies) in immune animals. Moreover, even a short exposure of stromal cells under these conditions (immune-to-normal variant) appreciably reduces these parameters in older (7 months) transplants. It seems that bone tissue reorganized under conditions of autoimmunity can be also defective by these parameters, which can be one of the mechanisms of osteoporosis associated with autoimmune diseases [9] and can lead to disorders in the microenvironmental functions of stromal tissue (and related disorders in hemo- and lymphopoiesis). It was shown that the capacity of the stromal sublayer to maintain the growth of CD34(+) cells was impaired in long-living cultures of bone marrow cells from patients with rheumatoid arthritis [10].

Immunization of animals with antigens leads to a sharp increase in the number of CFC-F in their lymph nodes and spleen [2]. It is noteworthy that the count of CFC-F in the femoral bone marrow from animals immunized with killed group A (type 5) streptococcus vaccine was also appreciably (3.5 times) higher than CFC-F content in femoral bone marrow in normal mice (Table 2). However, this high count was inessential for the size of the transplant formed from such bone marrow or for ECF-F and CFC-F values in this transplant. These data seem to indicate that not all CFC-F, whose count increases after treatment with antigens, are responsible for transplantability of the stromal tissue in case of heterotopic transplantation. Hence, the fact that bone marrow for transplantation is enriched with CFC-F does not yet ensure more effective formation of the transplant. It seems that the methods proposed for amplification the pool of stromal precursor cells used for

transplantation should be evaluated from the viewpoint of possibility of increasing the population of CFC-F responsible for transplantability.

In general, these experiments demonstrated considerable modification of the bone marrow stromal tissue under the effect of streptococcal infection and the relevant autoimmune reactions.

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