Counts of Stromal Precursor Cells in the Bone Marrow and Heterotopic Bone Marrow Transplants from Mice Immunized with Group A Streptococcus Antigens during Different Periods after Immunization

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 147, No. 1, pp. 78-81, January, 2009
Original article submitted April 9, 2008

The counts of stromal precursor cells in bone marrow transplants obtained from animals 2 months after their immunization with killed type 5 group A streptococcus vaccine drop almost 3-fold in comparison with transplants from normal donors. Six months after donor immunization, the count of stromal precursor cells in the transplants reaches the normal level. The count of stromal precursor cells in bone marrow transplants from normal mice transplanted to recipients 6 months after their immunization with killed streptococcus vaccine also virtually did not change in comparison with the counts in bone marrow transplants from normal donors transplanted to normal recipients. The weight and size of bone capsules of 6.5-month bone marrow transplants in intact recipients after transplantation from donors immunized 2 months before with killed type 5 group A streptococcus vaccine was 3-fold lower than in bone marrow transplants collected from intact donors. The content of stromal precursor cells in the femoral bone marrow of animals immunized with killed streptococcus vaccine was 2.5 time higher in comparison with the parameter in the femoral bone marrow of normal mice even 8 months after immunization. The results indicate a significant long-acting effect of streptococcal antigens on the bone marrow stromal tissue, specifically, on its osteogenesis potential.

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

Since bone tissue restructuring is a permanent process on-going throughout the entire lifespan of the individual, a part of bone tissue can be reorganized by stromal stem cells against the background of an infectious process. The question is: how the infectious process (often followed by autoimmunity state) modifies the stromal tissue and what are possible consequences (immediate and delayed) of these situations. Here we tried to clear it out by using the model of heterotopic bone marrow transplantation; donors and recipients were immunized with streptococcal antigens, because this infection is highly prevalent, on the one hand, and often provokes the development of autoimmunity, on the other. According to current concepts, the main cause of autoimmune reactions in infections caused by streptococcus are cross-reacting group A streptococcal antigens, common with tissue antigens of the heart, kidney, skin, thymus, and other human and animal organs [1,5,6]. We selected the heterotopic transplantation method for this study for the following reason: 2-3 weeks after transplantation of the bone marrow fragments under the renal capsule, a bone

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marrow organ gradually populated by hemopoietic cells is formed at the sate of transplantation from stromal precursor stem cells (CFC-F) responsible for transplantation capacity [4]. The status of this organ seems to reflect the process of new bone marrow organ formation under certain conditions. We previously showed that immunization with streptococcal antigens modifies the bone marrow stromal tissue [2]. For example, the count of CFC-F in normal bone marrow transplants implanted to recipients 8 days after their immunization with killed type 5 group A vaccine decreased by 4.5-6.5 times, depending on the graft age, in comparison with similar transplants in normal recipients. The content of CFC-F drops 2-fold in 7-month bone marrow transplants from animals, immunized with killed streptococcus vaccine collected 8 days after immunization and injected to intact mice in comparison with CFC-F content in bone marrow transplants from intact mice implanted to intact animals. It was also shown that the content of CFC-F in mouse bone marrow 8-12 days after immunization with streptococcal antigens was significantly (3.5 times) higher than CFC-F content in normal mouse femoral bone marrow. However, it is unclear, during which period the effect of immunization of streptococcal antigens on bone marrow stroma persists. The tasks of our study were as follows: to study the dynamics of CFC-F in bone marrow transplants during various periods after donor or recipient immunization with streptococcal antigens and to find out during which period the effect of immunization with streptococcal antigens on the CFC-F count in the bone marrow of immune mice was retained in comparison with intact animals.

MATERIALS AND METHODS

The experiments were carried out on CBA mice (2-3 months) and guinea pigs (4-5 months) from Kryukovo Breeding Center. Mice were immunized with killed type 5 group A streptococcus vaccine [2] in ascending doses for 3 weeks. The vaccine was intraperitoneally injected in 3 consecutive days in doses of 1 billion bacterial cells during week 1, 2 billion cells during week 2, and 3 billion cells during week 3. Immunization of animals (rabbits, mice) according to this protocol leads to the formation of antibodies to streptococcus antigens and of autoantibodies to antigens of various host tissues, the maximum production of autoantibodies occurring from day 7 to day 14 after the last injection of the antigens [1,3]. Autoantibodies to heart tissue antigens in the sera of immune mice were detected by the indirect immunofluorescent (IIF) method on bovine heart sections using FITC-labeled serum to mouse immunoglobulins (N. F. Gamaleya Institute of Epidemiology and Microbiology). The method of staining and interpretation of the results are described previously [6].

Heterotopic transplantation was carried out as follows. Half of the mouse femoral bone marrow content was transplanted under the renal capsule of animals as described previously [4]. The following donor-recipient combinations were studied: 1) normal — normal (N→N); 2) immune — normal (I→N); and 3) normal — immune (N→I). Bone marrow cell suspensions from mice and guinea pigs and mouse bone marrow transplants were prepared with a syringe as described previously [7]. Bone marrow and bone marrow transplant cells ($5\text{-}20\times10^5$) were explanted in flasks with 25 cm^2 bottom area in 5 ml culture medium (α-MEM) with 20% FCS (Paneco) with antibiotics (penicillin and streptomycin, 100 μg/ml each). Bone marrow cells (10^7) from guinea pigs irradiated in a dose of 60 Gy (^{60}Co, 10 Gy/min) were added to some cultures as a feeder. The cultures were incubated for 12 days at 37°C in a CO_2 incubator, after which they were fixed in ethanol, stained with azur and eosin, and colonies containing at least 50 fibroblasts were counted. Cloning efficiency (ECF-F; number of colonies formed by 10^5 explanted cells) was evaluated by the number of colonies.

RESULTS

The presence of autoantibodies in immune mouse sera was confirmed by IIF on bovine heart sections. Antibodies reacting with the myocardial myofibril sarcolemma and subsarcolemma antigens were detected. It was previously shown that these antibodies were directed to the organ-specific myocardial antigens common for animals of different species (rabbit, mouse, cow, *etc.*), in other words, they were autoantibodies [1,3]. Hence, immunization of animals according to the above protocol results in the production of not only antibodies to streptococcal antigens, but also autoantibodies. The content of nuclear cells in the transplants was virtually the same for all age groups at all the studied variants of transplantation (Table 1). ECF-F in cell cultures of bone marrow transplants and the counts of CFC-F decreased almost 3-fold in the I→N transplants in comparison with the N→N variant if the bone marrow for transplantation was collected 2 months after donor immunization with killed streptococcal vaccine and virtually did not change in comparison with the control if the bone marrow was transplanted 6 months after immunization (Table

TABLE 1. ECF-F of Bone Marrow Transplants from Normal Mice and Mice Immunized with Killed Group A Streptococcus Vaccine

Transplantation variant	Month after immunization	Age of transplant, months	Nuclear cell count per transplant, $\times 10^6$	ECF-F, $\times 10^{-5}$	Count of CFC-F in transplant
N→N	2	1	2.9±0.3	5.3±0.4	145±20
I→N			3.4±0.4	1.8±0.3	55±5
N→N	6	2	3.8±1.1	1.8±0.2	70±12
N→I			4.4±0.9	2.0±0.2	92±15
I→N			4.4±1.0	1.6±0.2	70±15

1). Six months after recipient immunization the ECF-F values and CFC-F content in the N→I bone marrow transplants virtually did not change in comparison with the N→N transplantation variant. These data seems to indicate that long (2 months) presence of stromal tissue in an immune organism leads to a drop (almost 3-fold) in the CFC-F count in the transplant organized by it; in other words, the transplant is defective by the ECF-F (concentration) and count of CFC-F. We previously showed that even short exposure of stromal tissue under these conditions (I→N variant) reduced significantly these values in transplants of delayed periods (7 months) [2]. The weight and size of bone capsules in bone marrow transplants collected 2 months after donor immunization with killed type 5 group A streptococcus vaccine and developing in intact recipients were 3-fold lower in the transplants aged 6.5 months in comparison with similar bone marrow transplants from intact donors (Table 2). These data suggest that the transplant capacity (capacity to construct a new full-value bone marrow organ and maintain normal CFC-F count in it) is impaired in the stromal tissue exposed to an infectious process or autoimmunity. This can be a mechanism of osteoporosis associated with infections and autoimmune diseases [8] and can lead to disorders in the microenvironmental functions of the stromal tissue and the concomitant disorders in hemo- and lymphopoiesis. The capacity of the stromal sublayer to maintain the growth of $CD34^+$ cell is impaired in continuous bone marrow cell cultures derived from patients with rheumatoid arthritis [9]. Only the bone marrow collected 6 months after immunization of donors with killed streptococcus vaccine can form a transplant with CFC-F count approaching the normal level in normal recipients. Only 6 months after recipient immunization, the transplanted normal bone marrow can provide the count of CFC-F, close to the normal value. Hence, immunization with

TABLE 2. Weight and Size of Bone Capsules in Bone Marrow Transplants from Normal Mice and Mice Immunized with Killed Group A Streptococcus Vaccine 2 Months before

Transplantation variant	Transplant fixation period, months	Nuclear cell count per transplant, $\times 10^6$	Bone capsule weight, mg	Proportion of bone capsule areas, N→N/ I→N transplants
N→N	1	2.9±0.3	0.3±0.02	1:1.2
I→N		3.4±0.4	0.4±0.05	
N→N	6.5	8.0±0.4	4.0±0.3	3:1
I→N		9.8±0.6	1.4±0.3	

TABLE 3. The ECF-F of Femoral Bone Marrow Cultures Derived from Normal Mice and Mice Immunized with Killed Group A Streptococcus Vaccine

Bone marrow donor	Month after immunization	Nuclear cell count per femoral bone, $\times 10^6$	ECF-F, $\times 10^{-5}$	Count of CFC-F per femoral bone
N	1.5-2	21.6±1.2	1.3±0.3	283±71
I		23.2±1.2	2.9±0.6	670±112
N	6-8	24.2±1.3	2.1±0.2	413±46
I		24.5±1.8	4.9±0.9	1128±234

streptococcus antigens has a lasting inhibitory effect on the transplantation capacity of stromal stem cells. We previously showed that CFC-F count in the femoral bone marrow collected 8-12 days after mouse immunization with killed type 5 group A streptococcus vaccine was significantly (3.5 times) higher than the CFC-F count in the femoral bone marrow of intact mice [2]. Here we showed that this increase (about 2.5 times) persisted for at least 8 months after immunization (Table 3). It was found that transplantation capacity of the bone marrow from immune donors, in which CFC-F content 2 months after immunization 2.4-fold surpassed the normal, was reduced significantly (CFC-F content in the transplants being almost 3-fold lower than normally) in comparison with normal bone marrow. This result is in line with our previous findings. In our previous study we showed that increased count of CFC-F in the bone marrow 8 days after immunization was inessential for the size of the transplant formed from this bone marrow or for the ECF-F value and CFC-F count in it [2]. It seems that these data one more time confirm that not all CFC-F, whose counts increase after stromal tissue exposure (immunization in our case), are responsible for the transplantation capacity of the stromal tissue in its heterotopic transplantation, and that the count of cells responsible for transplantation capacity of this pre-cultured population can even decrease. Hence, the presence of CFC-F in the bone marrow intended for transplantation is not a guarantee of more effective formation of the transplant. It seems that this fact should be taken into consideration when choosing the method for CFC-F pool enrichment for further transplantation.

Hence, the results indicate a lasting effect of streptococcal antigens on the bone marrow stromal tissue, specifically, on its osteogenesis capacity.

The authors are grateful to N. A. Borodiyuk, Doct. Biol. Sci., Laboratory of Immunity Regulation of N. F. Gamaleya Institute, for vaccine used in the study.

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