The "Graft Versus Leukemia" Effect under Conditions of Mixed Chimeric Hemopoiesis

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The "graft versus leukemia" effect can be induced in (C57Bl/6×DBA) F_1 mice by two transplantations of cell suspensions containing bone marrow cells and splenocytes of (CBA×C57Bl/6) F_1 mice and an injection of cyclophosphamide. The recipients of cell allotransplants were characterized by mixed chimeric hemopoiesis. These data outline a new approach to induction of the graft versus leukemia effect, requiring no complete elimination of the recipient's hemopoiesis and characterized by a low probability of lethal graft-versus-host reaction.

Key Words: *mixed chimeric hemopoiesis; graft versus leukemia effect*

Transplantation of allogenic bone marrow is the radical method of the treatment of leukemia. The antitumor effect of bone marrow allotransplant (graft versus leukemia effect) is due to the capacity of the transplant to suppress the development of leukemia from tumor cells remaining in the body despite stringent chemoradiotherapy before transplantation [1,4, 5]. Selection of a donor compatible with the recipient by the main histocompatibility complex impedes wide use of bone marrow allotransplantations. Standard transplantation of allogenic hemopoietic cells implies complete elimination of the recipient's hemopoiesis before transplantation and involves a high risk of complications (acute graft versus host reaction and severe immunodeficiency) leading to lethal outcome [3,4]. The results of this work outline a new approach to induction of the graft versus leukemia effect (GVLE) which does not involve complete elimination of the recipient's hemopoiesis before transplantation and is based on a combination of standard (nonlethal) cytoreducing therapy and transplantation of allogenic hemopoietic cells.

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MATERIALS AND METHODS

(C57Bl/6×DBA) F_1 (BDF_1, H-2^b/H-2^d) and (C57Bl/6×CBA) F_1 (CBF_1, H-2^b/H-2^k) mice aged 4-7 months from the breeding center of the Siberian Division of the Russian Academy of Medical Sciences were used. The animals were maintained on sterile ration and acidified (pH 2.8) water with antibiotics.

Mastocytoma P815 (DBA, H-2^d) cells were obtained from the Oncology Research Center, Russian Academy of Medical Sciences, and preserved as ascites in BDF_1 mice.

Experimental BDF_1 mice were subjected to the following procedures at two-day intervals: 1) intravenous injection of 7×10^5 P815 cells (6 days before examination); 2) intravenous transplantation of cell suspension containing bone marrow cells (25×10^6 per mouse) and splenocytes (55×10^6 per mouse) of CBF_1 mice (4 days before investigation); 3) intraperitoneal injection of cyclophosphamide (CP, cyclophosphane, Biokhimik Research and Production Unit, Saransk) in a dose of 200 mg/kg (2 days before study); and 4) repeated intravenous transplantation of bone marrow cells (25×10^6 per animal) and splenocytes (7×10^6 per mouse) of CBF_1 mouse (day 0). Control mice with tumors were either injected CP or CP+syn-

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geneic cell transplantation. Control group for the graft versus host reaction (GVHR) was subjected to procedures 2, 3, and 4. Each experimental group consisted of 10-14 animals. The cause of death was established after pathomorphological analysis.

The percentage of donor cells in bone marrow was estimated in the standard cytotoxicity test with allospecific antisera and low-toxic rabbit complement (Cedarline). Allospecific sera BDF_1 to CBA and CBF_1 to DBA reacting with donor and recipient cells, respectively, were obtained after multiple alloimmunizations [2]. The level of donor chimerism was calculated from the formula: $A/(A+B)\times 100$, where A and B are the counts of donor and recipient cells, respectively.

The results were processed using the Wilcoxon—Mann—Whitney's U test, and the differences were considered as significant at $p<0.05$.

RESULTS

A previously described [8-10] method for inducing mixed chimeric hemopoiesis in BDF_1 mice injected with P815 cells was used. It consisted in intravenous injection of 5×10^7 bone marrow cells+3×10^7 splenocytes of CBF_1 mice per animal and, two days later, injection of CP in a dose of 200 mg/kg. Previous studies [8,9] showed that the specific tolerance was due to CP-effected elimination of alloantigen-activated T lymphocytes. It was shown that a single cell allotransplantation together with CP did not significantly prolong the life span of mice with tumors. These data prompted us to alter the transplantation protocol to stimulate the donor hemopoiesis responsible for GVLE in the recipients. The new protocol included an additional transplantation of allogenic cells (25×10^6 bone marrow cells+7×10^6 splenocytes per mouse) 2 days after injection of CP. Figure 1 shows that a combination of two cell allotransplantations and CP significantly prolonged the life span of animals with tumors. It is noteworthy that

TABLE 1. Percentage of Donor Cells in the Bone Marrow of BDF_1 Mice Injected CP and Transplanted CBF_1 Cells

Animal No.	% of donor cells on day 100 after the second cell transplantation	M±m
1	10	
2	7	
3	12	13.2±4.5
4	15	
5	20	
6	15	

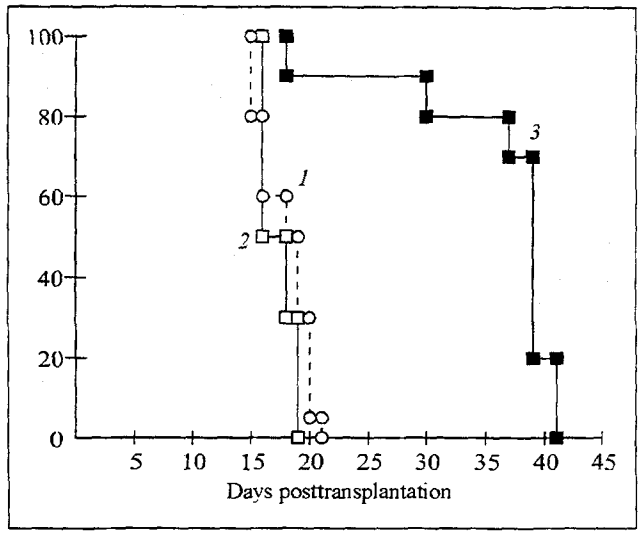

Fig. 1. Effect of cell allotransplantations on the life span of mice with tumors. Ordinate: BDF_1 survivors after injection of P815 cells and cyclophosphamide alone (*1*) and in combination with syngeneic (*2*) and allogenic (CBF_1) cell transplantations (*3*). Results of one experiment are shown.

transplantations of syngeneic cells performed according to the same protocol virtually did not prolong the life span of mice with tumors.

Cell allotransplantations combined with CP injections led to the development of stable mixed (auto+allo) hemopoiesis in the recipients. Table 1 shows that on day 100 after transplantation the content of donor cells in the bone marrow of control mice BDF_1, recipients of cells from CBF_1 mice, varied from 7 to 20%.

Hence, mixed hemopoiesis can be associated with the development of GVLE. This hypothesis is consistent with generation of GVLE in animals with tumors transplanted syngeneic and allogenic bone marrow cells after lethal irradiation [6,7].

The donor—recipient CBF_1—BDF_1 combination permits simulation of immunological processes that may occur in a recipient of related but HLA incompatible lymphohemopoietic transplant. We believe that for many reasons (one of which is the availability of donor material) bone marrow transplantations from relatives combined with cytoreducing therapy might be widely used in oncohematology. Interestingly, the presence of common antigens in the donor and recipient in our experiments apparently did not play the key role in the taking in of a hemopoietic transplant and development of GVLE. Pronounced GVLE directed against mastocytoma P815 was observed in DBA ($H\text{-}2^d$) mice transplanted cells from C57Bl/6 ($H\text{-}2^b$) mice according to the above protocol. However, in contrast to cells from C57Bl/6 mice, cells from CBA ($H\text{-}2^k$) or BALB/c ($H\text{-}2^d$) mice failed to generate GVLE in DBA mice (data

not shown). Therefore, a donor for generation of GVLE under conditions of mixed hemopoiesis should be selected proceeding not from the antigenic compatibility with the recipient, but from some other still unknown criteria.

Available data indicate that donor T lymphocytes are involved in induction of GVLE in recipients of a hemopoietic transplant [1,4,5]. The same cells are responsible for the development of acute lethal GVHR [1,4,5]. There is no distinct borderline between useful and harmful effects of T cell reactions, and this fact limits the clinical use of bone marrow transplantations. In our experiments more than 70% of control BDF_1 mice (injected no P815 cells) transplanted allogenic cells and injected CP survived throughout the entire follow-up period (5 months) without apparent signs of GVHR (data not shown). Thus, our results agree with the concept [3,6] that residual hemolymphopoiesis of a recipient can effectively impede the expansion of donor lymphopoiesis and thus prevent lethal GVHR.

The proposed approach to the treatment of leukemia holds good promise. By changing the dose of transplanted cells, number of transplantations, and parameters of cytostatic therapy it is possible to achieve the desired result and control untoward effects. The key factor is that the proposed scheme of transplantation does not involve total elimination of the recipient's hemopoiesis and does not require the HLA compatibility of the recipient and donor. Therefore, this method is prospective for the treatment of leukemia.

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