Synthetic Peptide Thymodepressin Promoted Take of Transplanted Hemopoietic Precursor Cells in the Bone Marrow of Irradiated Mice

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We studied the possibility of using a new synthetic heme-inhibiting peptide thymodepressin for improving the efficiency of transplantations of syngeneic and allogeneic bone marrow. Thymodepressin was injected to recipients 3 times (48, 24, and 2 h) before irradiation and transplantation of bone marrow suspension. The yield of 9- and 12-day colonies increased, the number of proliferating CFU-S-12 and pre-CFU-S in recipient bone marrow increased in comparison with the control. In case of an allogeneic donor-recipient combination, the number of 9-day splenic colonies in thymodepressin-treated animals increased 5-fold compared to the control. We hypothesized that thymodepressin therapy and irradiation form an adaptive response of the recipient hemopoietic microenvironment in the bone marrow, which provides conditions for optimal functioning of donor hemopoietic precursors.

Key Words: *splenic colony-forming units; hemopoietic microenvironment; synthetic peptide; thymodepressin*

Normal functioning of transplanted bone marrow cells in the recipient crucial for successful take is determined by the state of hemopoietic microenvironment maintaining viability of hemopoietic cells. Previous studies demonstrated that inhibitory peptide AcSDKP improved the efficiency of stem cell transplantation. This peptide is believed to affect homing of transplanted cells in recipient bone marrow [8].

Thymodepressin (TD) is a new virtually nontoxic peptide consisting of two dextrorotatory amino acid residues (D-iGlu-D-iTrp) and exhibiting immunosuppresor and heme-inhibiting properties comparable to those of AcSDKP. It suppresses splenic colony formation by intact bone marrow both *ex vivo* and *in vivo*; subsequent injection of intact thymocytes completely abolishes this effect. In proliferating bone marrow TD reduces the number of CFU-S in the S phase to a level of intact control [4], while injection of TD before irradiation in a dose of 4 Gy promotes recovery of CFU-S population in the bone marrow [1].

We investigated the possibility of using TD for improving the efficiency of syngeneic and allogeneic bone marrow transplantation.

MATERIALS AND METHODS

The study was carried out on female CBA, (CBA×C57Bl/6)F_1, (C57Bl/6×DBA)F_1 mice from Stolbovaya Breeding Center (Russian Academy of Sciences). The animals were kept under standard vivarium conditions on standard rations. TD synthesized at Peptos Engineering Center of Peptide Drugs was intraperitoneally injected to recipient mice in a dose of 25 mg/kg in 0.2 ml apyrogenic normal saline 48, 24, and 2 h before γ-irradiation (8 Gy, ^{60}Co, 1.5 Gy/min). Colony-forming capacity of the bone marrow was evaluated on days 9 and 12 after transplantation as described previously [9]. Sedimentation factor (f) was evaluated

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[7] and number of pre-CFU-S was determined [6]. The percentage of CFU-S-12 in the cell cycle S phase was evaluated by the thymidine suicide method [3]. The effect of TD on hemopoietic microenvironment was evaluated in experiments on lethally irradiated intact mice and TD-treated mice. Bone marrow from intact syngeneic donors was transplanted to all recipients. Four days after transplantation (before the start of intense proliferation of donor CFU-S in intact recipients) bone marrow was removed from primary recipients. An aliquot of bone marrow suspensions was incubated with ^{3}H-thymidine and then injected to lethally irradiated secondary recipients not treated with the peptide. The number of splenic colonies was evaluated on day 12 after transplantation.

The results were statistically processed using Student's *t* test.

RESULTS

Injection of TD to syngeneic recipients significantly increased the number of 9- and, especially, 12-day colonies (Table 1). The number of 12-day colonies increased by 65% compared to the control. In mice receiving 3 injections of TD before irradiation in a dose of 8 Gy, the number of endocolonies in the experimental group did not differ from the control. Precipitation of CFU-S-12 was the same in TD-treated mice and in controls (9.2 and 9.7%, respectively). Thus, TD had no effect on survival of endogenous CFU-S population and did not increase the percentage of donor colony-forming cells in the spleen. Pretreatment with TD 4-fold increased the number of CFU-S-12 in the femoral bone marrow compared to the control, the percentage of CFU-S-12 in S phase in the bone marrow of treated mice 3-fold surpassed that in the control (Table 2). It can be assumed that intensive proliferation of hemopoietic precursor cells is responsible for the increase of CFU-S-12 content in the bone marrow of TD-treated recipient mice.

Recovery of hemopoiesis after bone marrow transplantation is largely determined by survival and further maintenance of repopulating capacity of primitive precursor cells (pre-CFU-S). In light of this we evaluated the number of pre-CFU-S in the bone marrow of TD-treated recipients after transplantation of syngeneic bone marrow. First recipients were intact lethally irradiated and peptide-treated mice. On day 12 after transplantation, femoral bone marrow from first recipients was transplanted to lethally irradiated second recipients, and the number of colonies in the spleens were counted on day 12 after transplantation. It was found that the number of pre-CFU-S in the bone marrow of TD-treated primary recipients surpassed the control values.

In the next experimental series we evaluated the effect of TD in recipients receiving allogeneic bone marrow. CBA (H-2^k) were donors and BDF$_1$ (H-2^d) were recipients. Only 10% CFU-S survived in the spleen after transplantation of allogeneic bone marrow in comparison with syngeneic transplantation (Table 3). TD pretreatment almost 5-fold increased the number of 9-day colonies (more than 50% CFU-S survived). Hence, TD treatment promoted survival of

TABLE 1. Effect of TD Treatment on Survival of Hemopoietic Precursor Cells after Syngeneic Bone Marrow Transplantation (*M*±*m*, *n*=12)

Parameter	No treatment	Treatment
Number of exogenous CFU-S day 9	10.0±0.7	13.4±0.5*
day 12	9.8±0.8	16.9±0.6*
Number of pre-CFU-S	180±17	301±19*

Note. Here and in Table 2: *p<0.01 compared to untreated mice.

TABLE 2. Effect of TD Treatment on Proliferation of Hemopoietic Precursor Cells on Day 4 after Syngeneic Bone Marrow Transplantation (*M*±*m*, *n*=12)

Parameter	No treatment	Treatment
Number of exogenous CFU-S	11.6±1.0	46±5*
Including those in S phase	17.2	54.3

TABLE 3. Effect of TD Treatment on Formation of 9-Day CFU-S in Recipients Receiving Allogeneic (CBA) Bone Marrow (*M*±*m*, *n*=18)

Parameter	Recipient		
	CBA, no treatment	BDF$_1$	
		no treatment	TD treatment
Number of colonies/10^5 transplanted cells	10.8±0.4	1.2±0.1	5.7±0.3*
Percentage of survived CFU-S	100	11	53

Note. *p<0.001 compared to untreated BDF$_1$ recipients.

donor CFU-S in recipients receiving allogeneic bone marrow.

Hemopoietic microenvironment determines (via local regulatory factors) proliferation and maturation of hemopoietic cells in the recipient organism [2,10]. It can be assumed that successive TD treatment and irradiation forms an adaptive response of hemopoietic microenvironment in the recipient bone marrow and provide conditions for optimal function of donor hemopoietic precursors, mainly polypotent CFU-S-12. Intensive proliferation of CFU-S-12 probably ensures more complete recovery of hemopoiesis compared to intact recipients. There are data that pre-CFU-S are resting units and their expenditure is determined only by the need in differentiated precursors [5]. In view of this accumulation of pre-CFU-S in the bone marrow of syngeneic TD-treated recipients can be explained by a decrease in their expenditure.

In a syngeneic donor-recipient system, the peptide just negligibly affects the formation of 9-day colonies, while in allogeneic donor-recipient combinations the effect of TD on the number of 9-day colonies is much more potent. Hence, TD treatment before irradiation and transplantation of allogeneic bone marrow improves survival and further functioning of donor bone marrow stem cells, probably due to the effect on the hemopoietic microenvironment.

The problem of improving the efficiency of transplantation of syngeneic and, especially, allogeneic hemopoietic precursor cells is very important, as bone marrow transplantation is often used in the treatment of malignant diseases of the hemopoietic system. We demonstrated the possibility of using TD exhibiting heme-inhibiting activity for this purpose.

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