EXPERIMENTAL BIOLOGY

Dipeptide γ-d-Glu-d-Trp (Thymodepressin) Inhibits Migration of CD34+ Cells from the Bone Marrow into Peripheral Blood during Tumor Growth

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We studied the effect of dipeptide γ-d-Glu-d-Trp (thymodepressin) on migration of $CD34^+$ hemopoietic precursors and their direct adhesion to fibronectin in tumor-bearing mice on days 8, 11, 15, and 17 of tumor growth and on expression of CXCR-4 ($CD184^+$) to SDF-1 and integrin β_1 ($CD29^+$) by bone marrow cells. In tumor-bearing mice treated with γ-d-Glu-d-Trp, the percent of $CD34^+$ hemopoietic precursors in the peripheral blood considerably decreased throughout the observation period; the content of $CD34^+$ hemopoietic precursors in the tumor tissue was 2-3-fold below the control against the background of increased content of $CD34^+$ cells in the bone marrow. In animals treated with the peptide, the content of cells expressing CXCR-4 in the peripheral blood, bone marrow, and tumor tissue significantly decreased, while the percent of cells expressing integrin β_1 receptor ($CD29^+$) in the bone marrow increased 2-fold, which was paralleled by an almost 2-fold increase in the percent of cells binding to fibronectin. We hypothesized that dipeptide γ-d-Glu-d-Trp suppressed mobilization/migration of $CD34^+$ hemopoietic precursor cells from the bone marrow to the peripheral blood of tumor-bearing mice.

Key Words: *$CD34^+$ cells; γ-d-Glu-d-Trp dipeptide (thymodepressin); Lewis carcinoma; migration; adhesion*

Synthetic dipeptide γ-d-Glu-d-Trp (thymodepressin) consisting of artificial d-amino acid residues suppresses proliferation and differentiation of hemopoietic stem cells and reduces their migration from the bone marrow into the peripheral blood in healthy mice and in animals treated with granulocytic CSF. These properties are most likely determined by the effect of the peptide on receptor expression on hemopoietic precursors and accessory cells responsible for mobilization and homing, essential components of normal hemopoiesis [1-4]. Extremely unfavorable effects of increased migration of $CD34^+$ early hemopoietic precursors from the bone marrow into the peripheral blood on the growth and metastasizing of solid tumors were demonstrated: $CD34^+$ cells settled in the tumor tissue suppress cell immunity [9], promote tumor growth by activating angiogenesis [10], and initiate the formation of metastatic niches, which leads to enlargement of metastases [5].

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Here we studied the effect of γ-d-Glu-d-Trp dipeptide (thymodepressin) on migration and direct adhesion of $CD34^+$ hemopoietic precursors to fibronectin in tumor-bearing mice, expression of CXCR-4 receptors ($CD184^+$) to SDF-1 and integrin β_1 ($CD29^+$) promoting adhesion to fibronectin.

MATERIALS AND METHODS

Experiments were carried out on 48 female C57Bl/6 and $(CBA\times C57Bl6)F_1$ mice aging 2-3 months (body weight 20-22 g) obtained from Stolbovaya nursery. Lewis carcinoma was transplanted subcutaneously (into the right hindpaw) in a dose of 1.0×10^6 cells. The dipeptide γ-d-Glu-d-Trp (synthesized at the Department of Peptide Chemistry, Institute of Bioorganic Chemistry, Russian Academy of Sciences and Peptos Engineering Center of Peptide Preparations) was injected intraperitoneally in a dose of 500 μg/kg from day 5 (the mean tumor volume at this term was 200-100 mm^3) to day 20 of tumor growth. Control tumor-bearing mice received physiological saline according to the same scheme. Bone marrow samples (washed out from the femur with medium 199), peripheral blood and tumor tissue were taken on days 7, 11, 15, and 17 of tumor growth. Direct adhesion to fibronectin was evaluated by the previously described method [8] with some modifications: aliquots (5×10^5) of tumor or bone marrow cells were incubated on fibronectin-treated Nunc plates, unbound cells were removed. The number of attached cells was evaluated after methanol fixation and azure II staining (by optical density at λ=650). Binding with fibronectin in the experimental group was calculated as the percent from the control.

$CD34^+$-cells were identified in accordance with international recommendations (*i.e.* in the population characterized by low side scatter and weak expression of CD45) after staining of blood, bone marrow, and tumor cells with the corresponding monoclonal antibodies labeled with phycoerythrin and FITC (Pharmingen Becton Dickinson). The samples were analyzed on a FACS Vantage flow cytofluorometer (Becton Dickinson Immunocytometry Systems) equipped with Spectra-Physics 177-G1202 (Spectra-Physics) laser (λ=488 nm) within 2 h after staining. The data were processed using Cell Quest software (Becton Dickinson Immunocytometry Systems). The area corresponding to undamaged nucleated cells with low side scatter was determined on scatter intensity cell distribution diagram. The percent of $CD34^+CD45^{low}$ hemopoietic precursors among isolated cells was evaluated. The relative number of $CD29^+$ and $CD184^+$ cells was determined similarly. Nonspecific binding was controlled routinely using species-specific immunoglobulins (Caltag Laboratories) belonging to the same isotype and labeled with the same fluorochromes as the above specified antibodies to surface markers. Averaged results of 4 experiments (each group consisted of 12 animals) are presented.

The data were processed statistically using Student *t* test.

RESULTS

The relative number of $CD34^+$ precursors in the peripheral blood of control tumor-bearing mice considerably increased during tumor growth (to day 15); this increase was maximum on day 11 and was associated with an increase in the percent of these cells in the tumor tissue (maximum value was observed on day 17 of tumor growth, Fig. 1, *a, b*). The observed intensive migration of $CD34^+$ cells into the peripheral blood can be determined by increased level of cytokines, *e.g.* granulocytic-macrophage CSF produced by cells of the growing tumor [11]. In tumor-bearing mice receiving γ-d-Glu-d-Trp peptide, the number of $CD34^+$ hemopoietic precursors in the peripheral blood significantly decreased compared to the control at all terms of the experiment: on day 11, the number of $CD34^+$ cells was below the control by almost 4 times. The percent of $CD34^+$ hemopoietic precursors in the tumor tissue was by 2-3 times below the control on days 11-17 of tumor growth (Fig. 1, *a, c*). The decrease in the content of $CD34^+$ cells in the peripheral blood and tumor tissue was accompanied by their accumulation in the bone marrow (maximum on day 15 of tumor growth, Fig. 1, *b*).

These findings suggest that dipeptide γ-d-Glu-d-Trp suppresses migration of $CD34^+$ hemopoietic precursors from the bone marrow into peripheral blood in tumor-bearing mice, which leads to pronounced decrease in the content of these cells in the tumor node.

The processes of mobilization of hemopoietic precursor cells are largely determined by their adhesion interaction with extracellular matrix proteins and migration along the SDF1—CXCR4 gradient [6]. In this context, we studied the effect of γ-d-Glu-d-Trp peptide on the expression of receptors CXCR-4 ($CD184^+$) to chemokine SDF1 and integrin β_1 ($CD29^+$) responsible for adhesion to fibronectin and on direct adhesion of bone marrow and tumor cells to this protein on day 11 and 15 of tumor growth. In experimental animals receiving γ-d-Glu-d-Trp peptide, the content of cells expressing CXCR-4 in the peripheral blood increased on days 11 and 15, and

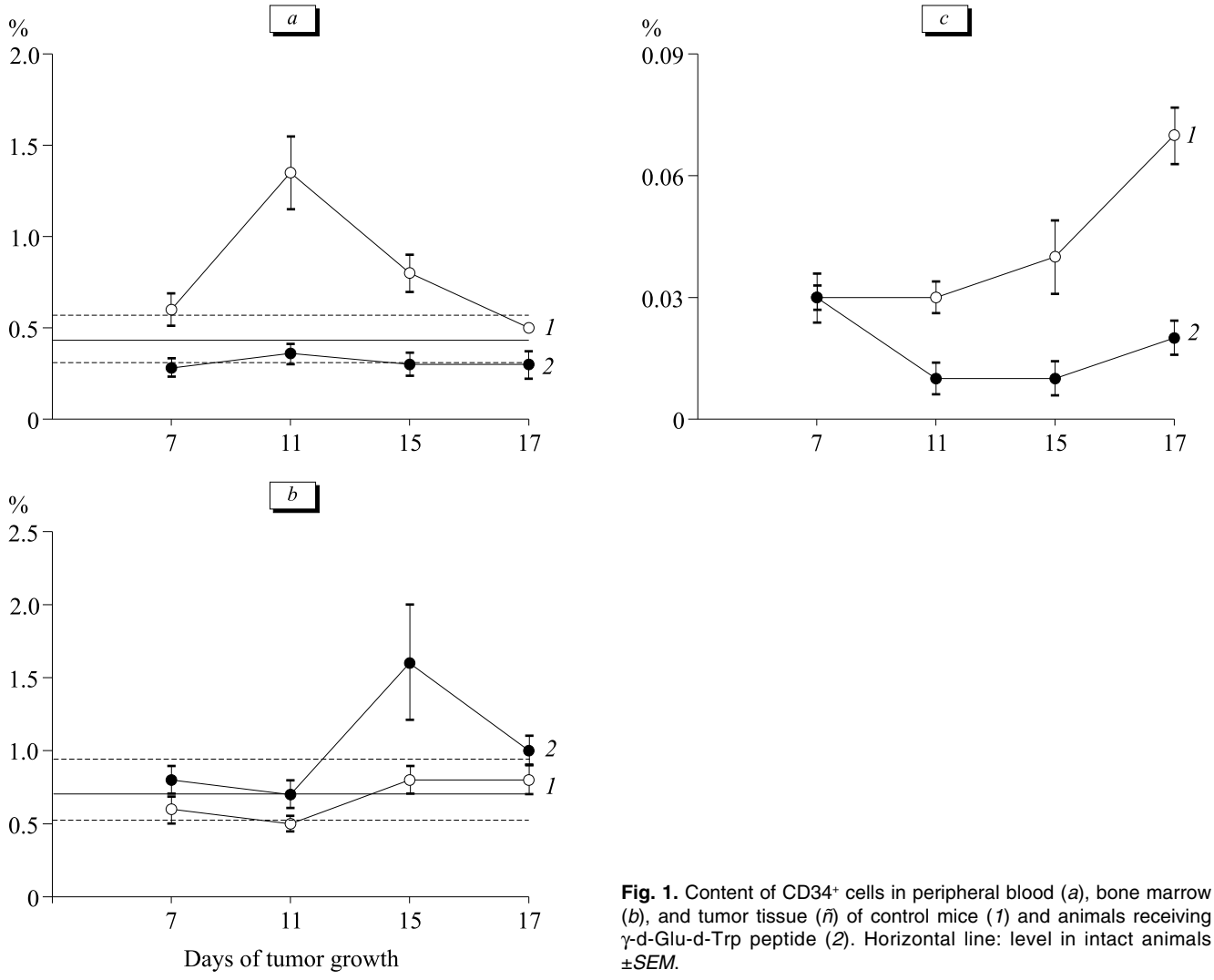

Fig. 1. Content of $CD34^+$ cells in peripheral blood (*a*), bone marrow (*b*), and tumor tissue (*c*) of control mice (*1*) and animals receiving γ-d-Glu-d-Trp peptide (*2*). Horizontal line: level in intact animals ±*SEM*.

in the bone marrow and tumor node on day 15 only (Table 1). Moreover, the percent of cells expressing receptors to integrin β_1 ($CD29^+$) in the bone marrow significantly increased on day 15 of tumor growth; in parallel, the percent of cells bound to extracellular matrix protein fibronectin increased 2-fold

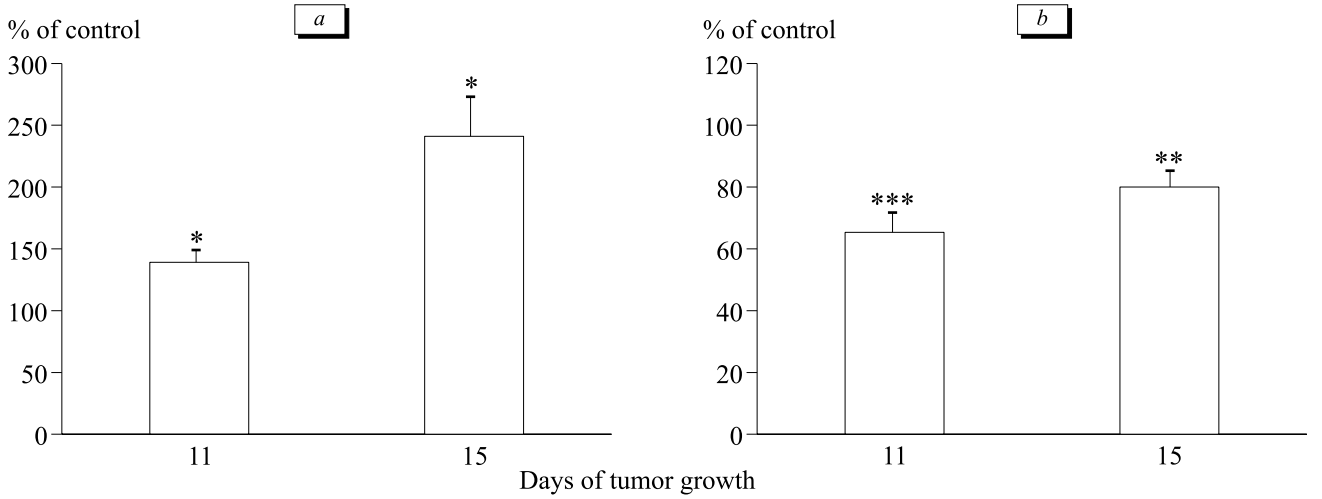

Fig. 2. Binding of bone marrow cells (*a*) and tumor cells (*b*) to fibronectin in mice treated with γ-d-Glu-d-Trp peptide. **p*=0.01, ***p*=0.004, ****p*=0.0006 compared to the control.

TABLE 1. Effect of Peptide γ-d-Glu-d-Trp on the Content of $CD184^+$ and $CD29^+$ Cells in Peripheral Blood, Bone Marrow, and Tumor Tissue of Tumor-Bearing Mice (%; *M*±*m*)

Day of tumor growth		Blood	Bone marrow		Tumor tissue	
		$CD184^+$	$CD184^+$	$CD29^+$	$CD184^+$	$CD29^+$
11	control	8.7±0.4	15.8±0.8	77.0±3.8	2.6±0.4	94.0±1.5
	peptide	5.6±0.4*	14.4±0.6	89.9±1.2*	2.3±0.06	90.0±1.3
15	control	13.8±0.4	15.4±0.4	59.0±3.2	5.5±0.5	85.0±2.2
	peptide	10.5±0.09*	11.6±0.3*	73.0±2.5*	3.2±0.2*	79.0±3.0

Note. *$p<0.05$ compared to the control.

(Table 1, Fig. 2). Treatment with γ-d-Glu-d-Trp significantly decreased the number of cells capable of direct binding to fibronectin, while the percent of cells expressing receptors to integrin β_1 ($CD29^+$) remained unchanged (Table 1, Fig. 2, *b*).

These findings suggest that γ-d-Glu-d-Trp suppresses mobilization of $CD34^+$ hemopoietic precursors from the bone marrow into peripheral blood in tumor-bearing mice due to enhanced binding of bone marrow cells to fibronectin and inhibition of their migration along the SDF1—CXCR4 gradient.

Since some properties of hemopoietic and tumor stem cells are similar (expression of common markers [7], similar migration mechanisms along the SDF1—CXCR4 gradient [6]), the presence of $CD34^+$ cells attests to unfavorable prognosis of the tumor process [11]. The observed capacity of γ-d-Glu-d-Trp peptide to inhibit migration of $CD34^+$ hemopoietic precursors in tumor-bearing mice can be used in the search and creation of new generation of drugs preventing metastatic dissemination of tumor cells.

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