Replacement of Accessory T Cells by Synthetic Peptides during the Formation of Splenic Hemopoietic Colonies

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UDC 612.419.017.1.08:612.112.91.017.1

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 116, № 9, pp. 298-299, September, 1993
Original article submitted March 22, 1993

Key Words: *T cells; synthetic peptides; splenic colony formation*

The discovery of a close relationship between the immune and hemopoietic systems is an important attainment of immunology. We have reported on a relationship between T cells and hemopoietic precursors and demonstrated that, along with CFU, thymocytes participate in colony formation in the spleen of mice exposed to lethal radiation, exerting control over CFU proliferation [1,4]. Investigation of studies of the mechanisms responsible for the regulation of the lymphocyte influence on hemopoiesis revealed a significant role of humoral factors such as lymphokines and immunoregulatory thymus peptides [2,3,9]. The latter include thymosins (fraction 5), thymopoietin, thymulin, etc. These peptides are the factors that determine the proliferation, differentiation, and function of T cells. However, there is evidence on the ability of thymus peptides to affect not only T cells but also other hemopoietic lines [8]. For example, we have shown that thymosin can restore colony formation inhibited by treatment of bone marrow with antiserum [3].

In this study we examined the regulatory effects of immunomodulating peptides on hemopoietic stem cells. For this purpose we used the thymogen Glu-Trp, a synthetic analog of the dipeptide purified from the thymus preparation thymalin; this peptide exhibits an immunoregulatory activity similar to that of the preparation [5]. We also employed the thymohexin Arg-Lys-Asp-Val-Tyr-Arg, which differs from the well-known preparation thymopeptin in the presence on an arginine residue (Arg) at the carboxyl end of the molecule.

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MATERIALS AND METHODS

Experiments were performed on (CBA×C57B1)F_1 hybrid female mice weighing about 20 g. The animals were irradiated in a Luch-1 radiotherapeutic apparatus (γ-irradiation of ^{60}Co, 1 Gy/min 18-24 hours prior to the injection of bone marrow suspension. Colony-forming activity was measured by the method of splenic exocolonies described elsewhere [10]. Rabbit anti-mouse brain serum (RAMBS) was prepared as described [7]. The bone marrow suspension was treated as previously indicated [1]. Thymocytes (2×10^7 cells per mouse) and peptides were injected intravenously 30-40 min prior to the bone marrow injection. Thymogen was injected intramuscularly at a dose of 10 μg/kg; thymohexin (T6) was injected intravenously in the same dose 30-40 min prior to bone marrow injection. Spleens were fixed in Bouin's fluid. Colonies were counted on day 9.

RESULTS

Upon testing the possibility of replacing the peptides accessory for T cell colony formation with their synthetic counterparts we employed the test system described previously [1]. In this system, T

0007-4888/93/0009-1138$12.50

TABLE 1. Effect of Peptides on Splenic Colony Formation after the Injection of RAMBS-Treated Bone Marrow Suspension ($M \pm m$)

RAMBS	Peptide, 10 mg/kg	Thymus, 2×10^7 cells/mouse	Number of mice	Mean number of colonies
–	–	–	20	10.5±0.7
+	–	–	20	2.0±0.4
+	–	+	19	7.0±0.5*
+	T6	–	16	7.1±0.8*
+	T6	+	18	3.0±0.5
+	Thymogen	–	16	3.2±0.6
+	Thymogen	+	17	6.1±0.5*

Note. Values significantly different from the control ($p<0.01$) are indicated with an asterisk.

cells that support the formation of splenic colonies are eliminated by RAMBS treatment and replaced with other factors. As shown previously, treatment of bone marrow with RAMBS results in a considerable decrease in the number of colonies restored by injection of thymocytes. In the present study T cells were replaced by either thymogen or T6. Thymohexin induced a 3-fold increase in the colony yield from RAMBS-treated bone marrow (Table 1), i.e., its effect was essentially similar to that of thymocytes. Such an effect was not observed after injection of thymogen. On the basis of these findings, we attempted to find out to what extent thymocytes and synthetic immunomodulatory peptides add to the action of each other upon interaction with CFU in this test system. To this end, thymocytes and one of the tested peptides were injected in lethally irradiated recipient mice. Thymocytes were injected 40 min and peptide - 20 min prior to the injection of bone marrow suspension. When administered together, T6 and lymphocytes lost the ability to restore colony formation by RAMBS-treated bone marrow. At the same time, thymogen did not inhibit the colony-stimulating activity of thymocytes (Table 1).

In an attempt to modify the experiment we either injected T6 with another concentration of thymocytes (10^7 cells per mouse), changed the injection mode - T6 was injected before, after or simultaneously (in one syringe) with bone marrow suspension - or preincubated thymocytes (4×10^7 cells/ml) with 1 μg/ml T6 (30 min at 37°C). In all cases the same result was obtained: co-administration of thymocytes and T6 did not restore the number of colonies that had been reduced after RAMBS treatment.

Our findings indicate that the factors (T cells and T6) having an accessory role in splenic colony formation compete with each other. This phenomenon may be caused by a nonspecific binding of the CFU surface receptors with thymocytes and peptide, which hampers normal cell functioning. However, our observations may have another explanation. It was found that the thymic peptide thymopoietin binds to the membrane receptors of T cells at two molecular sites and the animo acid sequences of one of these sites is identical to that of thymopentin [6], which is structurally similar to T6. It is likely that T6 binds to thymocytes and shields the receptor involved in the T cell-CFU interaction. It can be assumed that this receptor and T6 have a similar amino acid sequence, which may account both for the colony-stimulating activity of T6 comparable to that of thymocytes and for the lack of such activity in the case of thymogen whose two constituent amino acid residues are absent from the T6 molecule.

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