MICROBIOLOGY AND IMMUNOLOGY

Comparison of the Results of Interaction between Lymph Node-Derived Lymphocytes and Cortisone-Resistant Thymocytes, and Endogenous Stem Cells of Syngeneic and Allogeneic Recipients

T. V. Anfalova and N. I. Lutsan

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 119, № 6, pp. 637-639, June, 1995
Original article submitted June 16, 1994

A comparative study of the interaction between lymph node lymphocytes and cortisone-resistant thymocytes, and endogenous colony-forming units was conducted using an endogenous colony-forming assay. Cortisone-resistant thymocytes induced equally efficient stimulation of the spleen endocolonization in syngeneic, semiallogeneic, and completely allogeneic recipients. However, transfer of the lymph node lymphocytes resulted in only moderate stimulation of endogenous colony-forming units in syngeneic recipients and in drastic inhibition of colony formation in F_1 hybrid and allogeneic recipients. The mechanisms of the opposite effect of peripheral T lymphocytes as compared to cortisone-resistant thymocytes on endogenous stem cells are discussed.

Key Words: *stem cells; cortisone-resistant thymocytes*

The regulatory role of the thymus in the migration, proliferation, and differentiation of hemopoietic stem cells has been shown in many investigations [1]. However, the participation of individual thymocyte subpopulations in these processes has not been studied fully enough. Various subpopulations may exert different activities in the course of T-lymphocyte-mediated control of the function of hemopoietic stem cells. Cortisone-resistant thymocytes (CRT) represent a relatively homogenous, minor population of thymus cells which bear surface markers and realize immune reactions characteristic for mature T lymphocytes [6,7].

Research Institute of Carcinogenesis, Cancer Research Center, Russian Academy of Medical Sciences. (Presented by N. N. Trapeznikov, Member of the Russian Academy of Medical Sciences)

The goal of the present study was to compare the effect of lymph node-derived lymphocytes (LNL) and CRT upon the hemopoietic stem cells in the test of endogenous colony formation in syngeneic and allogeneic recipients.

MATERIALS AND METHODS

The experiments were carried out on mice of the CBA and C57Bl/6 strains, and (CBA×C57Bl/6) F_1 hybrids. For the generation of CRT, animals were injected intraperitoneally with 2.5 mg hydrocortisone per mouse 24-48 hours prior to sacrifice. In the test estimating endocolony growth, mice were subjected to sublethal radiation (500 R), and 8 days later the spleens were extracted and fixed with Bouin solution, after which the number of macrocolonies per spleen was counted. Statistical process-

0007-4888/95/0006-0614$12.50

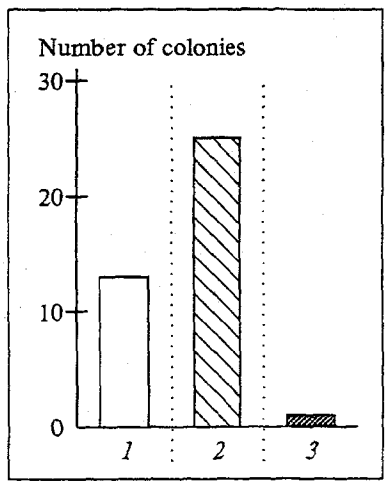

Fig. 1. Number of endocolonies formed in the spleen of (CBA×C57Bl/6) F_1 recipients pre-irradiated in a dose of 500 R after injection of CBA-derived CRT and LNL. Here and in Fig. 2: *1*) control; *2*) CRT (2×10^6 cells); *3*) LNL (2×10^6 cells).

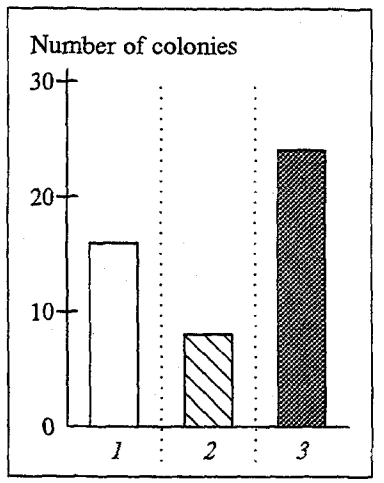

Fig. 2. Number of endocolonies formed in the spleen of CBA mice pre-irradiated in a dose of 500 R after injection of C57Bl/6-derived CRT and LNL.

ing included estimation of the arithmetic mean and standard error.

RESULTS

In the first experimental series the effect of LNL and CRT on colony formation in the syngeneic system was evaluated. For this purpose, CBA mice pre-irradiated in a dose of 500 R received 2×10^6 syngeneic cells. The results of two experiments are presented in Table 1. Transfer of CRT increased the number of endogenous colonies 2-3 times. Intact thymocytes transplanted in the same dose produced no effect on spleen endocolonization. Administration of LNL resulted in a slight increase in the number of endogenous colony-forming units.

Earlier it was shown that injection of parental CBA LNL to sublethally irradiated F_1 mice leads to the abolishment of endogenous colony growth in the spleen [2]. On the basis of the fact that CRT possess phenotypic and functional characteristics of mature T lymphocytes, one might suppose the action of these cells to be analogous to the lymph node T cells. However, the opposite results were obtained, i.e., transplantation of CBA LNL led to practically total inhibition of colony formation. CBA-derived CRT stimulated the spleen endogenous colony accumulation 2-fold (Fig. 1). The last results conflict with the data of another study [4], where it was simply noted that parental CRT do not inhibit spleen endocolonization in F_1 recipients.

In the last series of experiments we compared the effect of LNL and CRT on allogeneic stem cells. For this purpose, sublethaly irradiated CBA mice were injected with C57Bl/6 cells. CRT enhanced the endocolonization of the allogeneic spleen (Fig. 2). Transplantation of C57Bl/6 LNL to sublethally irradiated CBA recipients resulted in a drastic inhibition of colony formation. Thus, CRT enhance the endogenous colonization of the spleen in syngeneic and allogeneic donor-recipient combinations with equal efficacy.

Study of the mechanisms of nonsyngeneic stem cell inactivation by T lymphocytes has shown that effector T cells block hemopoietic stem cell proliferation during direct cell-to-cell contact [2,3], i.e., LNL exert a cytostatic effect on the colony-forming units. CRT lack this cytostatic potential and, moreover, they promote the growth of nonsyngeneic stem cells. This property, which distinguishes CRT from other mature T-lymphocyte subpopulations, may be connected with the functional peculiarities of the mentioned cells. CRT are

Table 1. Number of Endogenous Colonies Formed in CBA Mouse Spleens after Injection of Syngeneic LNL and CRT

No. of experiment	Control	Number of colonies after injection		Control	Number of colonies after injection of LNL
		intact thymocytes	CRT		
1	6.5±1.1 (11)	8.7±1.6 (9)	20±2.8 (9)	12.9±1.9 (10)	16.6±2.3 (10)
2	7.5±0.8 (9)	8.9±1.9 (10)	17.5±2.0 (9)	9.6±1.8 (9)	13.0±3.8 (9)

Note. The number of animals in a group is shown in parentheses.

resident thymus cells [6], with as yet unclarified functions. The regulatory effect of these cells on the hemopoietic stem elements is apparently mediated via humoral species-nonspecific factors produced by CRT. This hypothesis is supported by the data on the stimulation of colony formation by CRT culture supernatants [5]. Perhaps CRT produce both endogenous regulators of proliferation and chemotactic lymphokines which govern the migration of stem cells from the bone marrow to other lymphoid organs. An increase of the level of mediators that are secreted by CRT after transplantation to both syngeneic and allogeneic irradiated recipients boosts the entry of various hemopoietic precursors into the differentiation pool, thus leading to an increase in the number of endogenous spleen colonies.

REFERENCES

1. R. V. Petrov and V. M. Man'ko, *Itogi Nauki i Tekhniki, Ser. Immunologiya* [in Russian], Vol. 15, Moscow (1986), p. 109.
2. R. V. Petrov, R. M. Khaitov, and L. S. Seslavina, *Radiobiologiya*, **74**, № 10, 532 (1970).
3. R. M. Khaitov, *Byull. Eksp. Biol. Med.*, **74**, № 10, 58 (1972).
4. V. F. Semenkov and A. A. Kostyukov, *Ibid.*, **84**, № 7, 67 (1977).
5. T. V. Anfalova, N. I. Lutsan, and V. M. Man'ko, *Immunologiya*, № 4, 38 (1989).
6. O. Stutman, *Immunol. Rev.*, **42**, 138 (1978).
7. R. Boyd and C. Tucek, *Immunol. Today*, **14**, 445 (1993).