Formation of T Lymphocytes Inactivating Allogeneic Stem Cells in the Course of Macrophage-Thymocyte Interaction

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny,* Vol. 121, № 3, pp. 298-300, March, 1996
Original article submitted March 6, 1995

Allogeneic stem cells interact with T lymphocytes which are formed when intact thymocytes are cultured with syngeneic mononuclear phagocytes. The capacity of these T cells to inhibit the colony-stimulating activity of stem elements in endo- and exocolony formation tests is demonstrated.

Key Words: *macrophages; thymocytes; stem cell*

Macrophages have been shown to exert different effects on the function of the lymphoid system and to be involved in the antigen-dependent differentiation of T lymphocytes [1,6,8]. Previous reports [1,2] showed that short-term co-culturing of intact thymocytes with syngeneic macrophages leads to functional maturation of thymic cells, this manifesting itself in their acquiring the capacity to induce the graft-versus-host reaction (GVHR). The target cells of the regulatory effect of macrophages are hydrocortisone-sensitive immature cells of the cortical layer of the thymus. The reaction was assessed in the local GVHR test from the index of enlargement of the lymph nodes of F_1 recipients after administration of parental cells, and the result of the interaction between immunocompetent donor T cells and host T lymphocytes was evaluated.

Stem cells of the host are also known to act as targets in induced GVHR. We deemed it interesting to assess the capacity of T lymphocytes formed as a result of macrophage interaction with thymocytes to inactivate nonsyngeneic stem cells. The phenomenon of inactivation of allogeneic stem cells observed both in the exogenous and endogenous colony-formation system was used as the experimental system [4].

This paper describes the results of experiments in which we assessed the effects of interactions of allogeneic stem cells with the T lymphocytes which are formed when mononuclear phagocytes are cultured with intact thymocytes.

MATERIALS AND METHODS

CBA, C57Bl/6, and (CBA×C57Bl/6) F_1 mice were used in the study. A monolayer of peritoneal exudate macrophages was prepared as described previously [2]. Suspensions of thymocytes and bone marrow cells were prepared routinely.

Intact thymocytes of CBA mice were incubated with syngeneic macrophages for 18 h at 37°C. After culturing the cells were collected, washed, and administered to irradiated recipients.

In order to assess the endocolonies, F_1 mice were irradiated in a dose of 500 R 24 h before the cells to be analyzed were implanted. Exogenous colony formation was assessed on days 7 and 13 after transplantation of bone marrow cells of C57Bl/6 alone or together with thymocytes of CBA and F_1 mice exposed to a dose of 850 R. Then the spleens were removed and fixed in Bouin's fluid, and the number of macrocolonies was counted.

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0007-4888/96/0003-0274$15.00

TABLE 1. Suppression of Endocolony Formation in the Spleen of (CBA×C57Bl/6) F_1 Mice by Thymocytes of CBA Mice Co-Cultured with Syngeneic Macrophages

Experiment No.	Number of colonies in the spleen of (CBA×C57Bl/6) F_1 mice after transplantation of:			Inactivation index, %
	control	intact thymocytes	thymocytes and macrophages	
1	8±4 (6)	9.5±4.5 (6)	3.3±1 (6)	59
2	5.7±1 (10)	7.4±1 (9)	3.6±0.8 (9)	36
3	11.1±4 (8)	5±2.9 (6)	1.25±1 (7)	98
4	10.5±2.1 (10)	-	5.1±1 (10)	51

Note. In all experiments (CBA×C57Bl/6) F_1 mice irradiated in a dose of 500 R were transplanted 2×10^6 intact thymocytes or 2×10^6 thymocytes of CBA mice incubated with macrophages of CBA mice. Here and in Tables 2 and 3: the number of animals per group is shown in parentheses.

The results were statistically processed by calculating the arithmetic mean and standard errors. The effector function of T lymphocytes was characterized by the index of inactivation: $I=S-S_o/S$, where S is the mean number of colony-forming units in the control group and S_o is the number in the experimental group.

RESULTS

Khaitov [5] described a new form of local GVHR which manifests itself in depression of endogenous colony formation in the spleen of F_1 mice transplanted parental lymphoid cells. Using this test, we compared the effects of intact thymocytes of CBA mice and of thymic cells which interacted with macrophages on the endocolonization of the spleen in F_1 recipients on day 7 after exposure to a dose of 500 R. Table 1 shows that the index of inactivation due to the T cells which formed as a result of interaction with macrophages ranges from 36 to 98%. Intact thymocytes either did not affect the number of colonies, or (experiment 3) the reduction was far less than after transplantation of GVHR T effectors.

The next step was to assess the capacity of analyzed T lymphocytes to inactivate nonsyngeneic stem cells upon exogenous administration of bone marrow cells. To this end, F_1 mice irradiated in a dose of 850 R were transplanted 3×10^5 bone marrow cells from C57Bl/6 mice alone or in a mixture with 2×10^6 thymocytes of CBA mice. Macrocolonies were counted on day 7 after cell grafting. The data presented in Table 2 indicate that joint transplantation of thymocytes cocultured with macrophages and of allogeneic bone marrow cells leads to 50% inactivation of colony formation. In this dose intact thymocytes of CBA mice did not affect the colony-forming capacity of the graft.

Colonies of hemopoietic cells are believed [7] to form two types of precursors in the spleen of irradiated recipients: "early" colonies (7-8 days) are formed mainly by committed precursors, "late" colonies (10-13 days) by pluripotent stem cells capable of differentiating in different directions of hemopoiesis. Table 3 presents data on the capacity of analyzed T lymphocytes to affect the formation of "late" exogenous colonies (13 days). T lymphocytes formed as a result of interaction between thymocytes and macrophages inactivate "late" hemopoietic splenic colonies by 98%, that is, T cells which mature under the influence of macrophages from areactive thymocytes are equally effective in suppressing the formation of both committed precursors and pluripotent stem elements.

TABLE 2. Suppression of Colony Formation in the Spleen of (CBA×C57Bl/6) F_1 Recipients with Thymocytes of CBA Mice Incubated with Syngeneic Macrophages

Experiment No.	Number of colonies in the spleen of (CBA×C57Bl/6) F_1 mice after transplantation of:		
	bone marrow cells	bone marrow cells and intact thymocytes	bone marrow cells and thymocytes preexposed to macrophages
1	24.1±2.4 (10)	-	14±2.3 (10)
2	23±2.2 (8)	24.4±4 (8)	-
3	22.5±2.1 (10)	21±1.9 (10)	11.3±2.3 (11)
4	19.6±1.7 (13)	18±1.4 (11)	8±2 (7)
5	25.2±4.7 (8)	25±1 (7)	10.1±2.2 (9)

Note. Here and in Table 3: in all experiments (CBA×C57Bl/6) F_1 mice irradiated in a dose of 850 R were transplanted 3×10^5 bone marrow cells of C57Bl/6 mice alone or together with CBA thymocytes which were incubated with syngeneic macrophages.

TABLE 3. Suppression of Late Colony Formation by CBA Thymocytes Cultured with Syngeneic Macrophages

Experiment No.	Number of colonies in the spleen of (CBA×C57Bl/6) F_1 mice after transplantation of:		
	bone marrow cells	bone marrow cells and intact thymocytes	bone marrow cells and thymocytes preexposed to macrophages
1	14.1±1 (3)	4.1±2.2 (7)	0.9±0.4 (7)
2	20.2±1.2 (7)	-	0.4±0.2 (8)
3	12.4±2.6 (8)	6.8±1.6 (6)	0.75±0.4 (6)

The inactivating activity of T cells formed in the course of thymocyte-mononuclear phagocyte interactions differs from the inhibitory effect of peripheral T lymphocytes. When transplanted in the same dose (2×10^6), lymphocytes from lymph nodes virtually completely suppress "early" colony formation [3] in comparison with the 50% inactivation caused by T lymphocytes forming from areactive thymocytes. But both populations of lymphoid cells possess the same cytostatic potential toward "late" colonies; in other words, polypotent stem cells are more sensitive to the inhibitory effect of allogeneic T lymphocytes.

Hence, interaction between macrophages and subpopulations of immature T-effector precursors localized in the thymus cortex is conducive to their functional maturation; T lymphocytes are formed which alter colony formation when transplanted to recipients. Our experiments demonstrate the capacity of formed T cells to react directly with hemopoietic stem cells by inhibiting the colony-forming activity of stem elements in tests of endo- and exogenous colony formation.

It should be noted that different populations of effector cells may form in the course of coculturing of macrophages with thymocytes. One of the problems remaining to be studied is whether the same T lymphocytes react with allogeneic T cells and colony-forming units, or whether this is a result of the functional activity of different effector cell populations.

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