USE OF ANTILYMPHOCYTIC SERUM TO DETERMINE THE TIME REQUIRED FOR LYMPHOCYTES TO INACTIVATE ALLOGENEIC STEM CELLS

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In systems of exogenous or endogenous colony formation interaction between lymphocytes and genetically foreign hematopoietic stem cells is accompanied by inactivation of the endogenous or exogenous, allogeneic colony-forming units (CFU). Injection of antilymphocytic globulin (ALG) simultaneously with transplantation of the cell suspension into irradiated recipients or within 30 min thereafter abolishes the inactivating action of the lymphocytes. An increase in the time interval between injection of the cells and ALG to 1-2 h has the result that the preparation no longer affects the ability of the lymphocytes to block proliferation and differentiation of the foreign CFU. The main events leading to inactivation of allogeneic stem cells by the lymphocytes evidently take place in the course of 1-2 h.

It was shown previously that transplantation of a mixture of genetically foreign cells of hematopoietic and lymphoid tissues into lethally irradiated recipients is followed by inactivation of the colony-forming units (CFU) of the graft under the influence of allogeneic lymphocytes in the mixture [5-8]. Injection of antilymphocytic serum (ALS) into the recipients abolishes the inactivating action of the lymphocytes without any damage to the colony-forming ability of the stem cells [4, 6].

The period of time required to produce inactivation of allogeneic CFU by lymphocytes was determined in the present investigation with the aid of ALS. In a parallel series of experiments the action of ALS on colony-forming stem cells was investigated at the various stages of colony formation.

EXPERIMENTAL METHOD

C57BL mice were used as donors of bone marrow cells and CBA mice as donors of lymphocytes. The recipients were (CBA × C57BL)F_1 mice irradiated with γ-rays in doses of 850 or 600 R (438 R/min). Intravenous injections of $2 \cdot 10^5$ C57BL bone marrow cells and 10^6 CBA lymph gland cells were given to the recipients 24 h after irradiation in a dose of 850 R. Mice irradiated in a dose of 600 R received an intravenous injection of 10^6 CBA lymph gland cells only. The control animals received no cells (600 R) or an injection of bone marrow cells (850 R) only. The recipients were sacrificed 10 days after irradiation, the spleens were removed and placed in Bouin's fixative, and the number of colonies was counted and their size measured [3]. Antilymphocytic γ-globulin (ALG), isolated from horse ALS, was injected into the recipients simultaneously with the transplanted cells or 30 min to 5 days later. The methods of obtaining ALG and the characteristics of the preparations used were described previously [1,2].

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TABLE 1. Effect of Time Interval between Transplantation of Cells and Injection of ALG on Colony Formation in Spleen and Ability of Lymphocytes to Inactivate Nonsyngeneic Stem Cells (M ± m)

Time interval between transplantation of cells and injection of ALG	No. of colonies after injection of ALG	Diameter of colonies grown (mm)	No. of colonies after injection of CBA lymphocytes + ALG	Diameter of colonies grown (mm)
System of exogenous colony formation*				
ALG not injected	9,6±0,9 (46)†	0,91±0,02	0,4±0,1 (36)†	0,58±0,07
10—20 sec	25,6±1,7 (16)	1,03±0,03	24,8±1,8 (41)	0,98±0,01
1 h	—	—	0 (5)	—
3 h	25,3±0,2 (25)	1,31±0,02	0,4±0,2 (16)	0,49±0,08
6 h	18,8±1,3 (9)	1,25±0,03	0,5±0,5 (2)	0,3
12 h	22,2±1,4 (7)	1,19±0,04	0 (2)	—
1 days	10,6±0,9 (10)	1,15±0,02	0 (7)	—
3 days	9,5±1,0 (9)	0,99±0,03	0 (10)	—
5 days	9,2±1,2 (11)	0,67±0,03	0 (12)	—
System of endogenous colony formation‡				
ALG not injected	14,9±1,4 (19)	1,08±0,03	0,9±0,3 (28)	0,77±0,10
10—20 sec			22,3±1,7 (21)	1,15±0,03
30 min	33,8±3,0 (9)	1,32±0,03	21,1±2,3 (10)	1,11±0,03
1 h			8,7±1,3 (9)	0,81±0,08
3 h			1,4±0,2 (11)	0,96±0,10
5 days	22,2±2,5 (10)	0,74±0,04	1,3±0,5 (7)	0,92±0,39

*ALG (31.7-41 mg protein/ml) was injected intraperitoneally into recipients in a dose of 0.2 ml per mouse from 10-20 sec to 5 days after transplantation of $2 \cdot 10^5$ C57BL bone marrow cells (control) or $2 \cdot 10^5$ C57BL bone marrow cells and 10^6 CBA lymphocytes (experimental).

† Here and subsequently the number of animals used is shown in parentheses.

‡ ALG (31.7-41 mg protein/ml) was injected intraveously into recipients in a dose of 0.2 ml per mouse 1-6 days after sublethal irradiation (control) or 10-20 sec to 5 days after transplantation of 10^6 CBA lymphocytes (lymph gland cells of CBA mice were injected into recipients 24 h after sublethal irradiation).

EXPERIMENTAL RESULTS

As Table 1 shows, combined transplantation of lethally irradiated mice with C57BL bone-marrow cells and CBA lymphocytes was followed by the virtually complete inactivation (95.8%) of the CFU of the graft and by inhibition of growth of the uninactivated (4.2%) stem cells: the mean size of the growing colonies was reduced by more than one-third. Intraperitoneal injection of ALG simultaneously with transplantation of the cell mixture abolished the blocking action of the lymphocytes. An increase in the time interval between transplantation of the cell mixture and injection of the ALG to 1 h or more had no further effect on the inactivating action of the lymphocytes: the blocking of CFU proliferation was complete (100%). After transplantation of bone marrow cells only into the recipients, ALG stimulated colony formation and proliferation of the CFU (10-20 sec to 12 h) or had no effect on these processes (1-5 days). The same results in principle were recorded after intravenous injection of ALG, whether in the system of exogenous or of endogenous colony formation.

The possibility cannot be ruled out that immediately after transplantation of the cell mixture irreversible interaction took place between the lymphocytes and the foreign stem cells, leading to death of the CFU. If the ALG was injected before the critical stage of that process, cessation of inactivation of the allogeneic CFU was observed. However, the ALG no longer influenced this process if interaction between the cells took place before its injection. The ineffectiveness of delayed injections of ALG may be explained also by the fact that antilymphocytic preparations have a weaker action on lymphocytes in the tissues than on circulating lymphocytes [9, 10]. Since the delayed injection of ALG did not affect the ability of the lymphocyte to inactivate allogeneic CFU, but still stimulated colony formation, the CFU presumably must circulate longer in the blood than the lymphocytes. In other words, colonization of the tissues by stem cells may take place against the background of lymphocytes "established in fixed positions" and no longer accessible to the action

of ALG. In that situation interaction between the cells may also take place at longer time intervals after transplantation of the cell mixture, but just as in the preceding case, this process is not affected by ALG. The unequal action of ALG on lymphocytes and CFU may be attributable to differences in the structure of the surfaces of the stem cells and lymphocytes.

However, regardless of the mechanisms of these phenomena taking place after transplantation of a mixture of allogeneic cells into recipients it is clear that during the 1-2 h elapsing after transplantation of the cell mixture the principal events leading to inactivation of allogeneic CFU by lymphocytes take place.

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