EFFECT OF HEMATOPOIETIC STEM CELL INHIBITORY FACTOR ON DEVELOPMENT OF THE IMMUNE RESPONSE

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The writers showed previously that lymphocytes activated by antilymphocytic globulin (ALG) liberate a soluble factor (stem cell inhibitory factor, SCIF), which depresses the ability of bone marrow and spleen stem cells to form hematopoietic colonies [2, 4, 7]. Considering that mediators of cellular immunity (lymphokines) play an important role in the development of the immune response [2, 3, 6, 10, 13] it was decided to study the effect of SCIF on the immunologic competence of bone marrow B cells and splenic T and B cells.

This paper describes an attempt to study the effect of SCIF on the ability of bone marrow and spleen cells, when treated with the factor in vitro, to induce an immune response in an adoptive system.

MATERIAL AND METHOD

Experiments were carried out on (CBA × C57BL)F_1 hybrids. ALG was isolated from an antilymphocytic serum, for example, by immunizing rabbits with spleen cells of CBA mice [1, 7]. SCIF was obtained as follows: thymus cells (4×10^7 cells/ml) were treated in vitro with ALG in a concentration of 5 mg/ml for 30 min at 37°C, then washed three times with medium 199 and incubated in "pure" medium for 2 h at 37°C. The resulting supernatant, containing SCIF, was tested for its ability to inhibit clone formation by bone marrow cells in an exocolonization system [12]. Supernatant from thymocytes treated in the same way with normal rabbit globulin (NRG) was used as the control. Immunochemical analysis ruled out the presence of ALG eliminated from the surface of the treated cells in the supernatant [4, 7].

When the effect of SCIF on immunologic activity of lymphocytes was studied, bone marrow cells (10^7 cells per mouse) were transplanted intravenously into lethally irradiated recipients (900 rads) together with thymus (5×10^7) for spleen cells (10×10^7). Simultaneously with bone marrow and thymus cells, the recipients received 2×10^6 sheep's red blood cells, followed by a further 5×10^8 cells 4 days later. The spleen cells were injected together with 5×10^8 sheep's red blood cells 4-6 days after irradiation. In the experimental groups, the spleen and bone marrow cells were treated in vitro before transplantation for 1 h with SCIF at 37°C and then washed with medium 199. In the control groups, intact cells or cells treated in the same way with supernatant of NRG or medium 199 were used for transplantation. On the 8th day after transplantation of bone marrow cells + thymus cells and on the 4th day after transplantation of spleen cells the number of antibody-forming cells (AFC) was determined by Jerne's method in the recipients' spleens.

EXPERIMENTAL RESULTS

The results of three experiments reflecting the ability of bone marrow cells treated in vitro with SCIF to develop an immune response to sheep's red blood cells when injected together with thymus cells are summarized in Table 1 in groups 1-6.

For instance, after transplantation of bone marrow cells and thymus cells from syngeneic donors separately into lethally irradiated recipients virtually no immune response was found in the recipients, whereas combined injection of these cells led to considerable AFC formation (Table 1, groups 1-3). Preliminary treatment of the bone marrow cells intended for transplantation with SCIF led to a decrease in the number of AFC. Similar treatment of the bone marrow cells with NRG supernatant had no effect on the ability of the

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TABLE 1. Effect of SCIF on Immunologic Reactivity of B and T Lymphocytes

Group No.	Transplanted cells: bone marrow, 10^6	Transplanted cells: thymus, $5 \cdot 10^7$	Transplanted cells: spleen, $10 \cdot 10^7$	Reagent used to treat the cells	Number of animals	Number of AFC in spleen: M (geometric mean)	Number of AFC in spleen: confidence interval at $P < 0.05$ level
1	+	—	—	—	18	25	<57
2	—	+	—	—	10	15	<38
3	+	+	—	—	18	2929	1935— 4 208
4	+	+	—	Medium 199	7	2389	870— 5 248
5	+	+	—	NRG supernatant	20	2526	1738— 3 388
6	+	+	—	SCIF	21	561	381— 812
7	—	—	+	—	7	7905	5248—11 481
8	—	—	+	Medium 199	22	6728	4365—10 000
9	—	—	+	NRG supernatant	24	6364	4570— 8 709
10	—	—	+	SCIF	25	625	457— 912

Legend. SCIF) Supernatant obtained after treatment of thymus with ALG; NRG supernatant) control supernatant obtained after treatment of thymus cells with NRG; AFC) antibody-forming cells.

latter to cooperate with intact thymus cells, and the level of the immune response in this case was the same as in the control (Table 1, groups 4-6).

Groups 7-10 in Table 1 summarize the results of four experiments to study the effect of SCIF on the ability of the spleen cells to develop an immune response to sheep's red blood cells.

On the 4th day after irradiation and intravenous transplantation of spleen cells and antigens, 7905 AFC were formed per spleen in the recipients. Preliminary treatment of the spleen cells with SCIF led to a sharp decrease in the number of AFC in the spleens of the recipients compared with groups for which the cells for transplantation were treated with NRG, or the spleen cells were incubated in medium 199 (Table 1, groups 8-10).

Preliminary treatment of the bone marrow cells with SCIF thus inhibited the immune response to sheep's red blood cells by about 80% compared with the control (Table 1, groups 5 and 6), but similar treatment of spleen cells with this factor reduced the number of cells by an order of magnitude (Table 1, groups 9 and 10). The fall in the level of the immune response, it can be tentatively suggested, was due to the following causes: a change in the pathway of migration of the bone marrow cells under the influence of SCIF, inactivation of B cells by repression of B precursors, disturbance of the ability of mature B cells to interact cooperatively with T cells, elimination of macrophages from the pool of cooperating cells, or liberation of secondary inhibitory factors by the macrophages. Analysis of the data suggests, however, that the main agent responsible for the action of SCIF is the B cell. Considering that a sharper fall in the level of the immune response was observed when spleen cells were treated with SCIF, mature descendents of B cells which had already succeeded in proliferating were more likely to be exposed to the action of the factor. Spleen tissue, as we know, is a complex cell system whose main function as the organ of immunity is cooperation between different populations of T, B, and A cells [5, 8, 9]. It has been shown that the distinguishing properties of the A cell population are their high radioresistance and preservation of their ability to restore the immune response during the first 48 h after irradiation. If, therefore, the macrophages were exposed to the action of SCIF, when transplanted spleen cells were subjected to the action of SCIF this evidently should not be reflected significantly in the development of the immune response because of compensation of the level of activity of the splenic A cells of the irradiated recipient. Meanwhile, the suggestion that monokines of some form are liberated requires further study.

The role of T helper and T suppressor factors, belonging to the group of "immunoregulatory" lymphokines, in the induction of the immune response is being widely discussed at the present time [2, 3, 10, 13]. It is also suggested that the category of effector T cells includes a subpopulation of T lymphocytes producing mediators of cellular immunity in response to stimulation by specific antigens [2, 8]. The possibility cannot therefore be ruled out that the action of SCIF is aimed at the splenic T cells which, in turn, can produce a certain mediator which inhibits the immune response. The fact that immunogenesis is inhibited by SCIF, as has been established, is thus evidence of the complexity of interaction between cells during development of the immune response and also of the indirect nature of the immunodepressive action of ALG.

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