5. Z. M. Mikhailova, and G. A. Mikheeva, The Immunobiological Reactivity of the Child [in Russian], Moscow (1974).
6. G. I. Podoprigora, Byull. Éksp. Biol. Med., No. 6, 706 (1977).
7. G. I. Podoprigora and V. A. Dushkin, Rules for Work with Gnotobiological Isolators (technical instructions) [in Russian], Moscow (1977).
8. C. Koch, Acta Pathol. Microbiol. Scand., Section B, 82, 136 (1974).
9. J. Sterzl, J. Rejnek, and J. Travnicek, Folia Microbiol. (Prague), 11, 7 (1966).
10. J. Travnicek, L. Mandel, A. Lanc, et al., Csl. Fysiol., 15, 240 (1966).

EFFECT OF STEM CELL INHIBITION FACTOR AND MACROPHAGE MICRATION INHIBITION FACTOR ON EXOCOLONIZATION AND MIGRATION OF MOUSE SPLEEN CELLS

E. B. Mysyakin, A. P. Suslov, T. A. Golovanova, and D. R. Kaulen

UDC 612.411.014. 2:612.6]-064

Hematopoietic stem cell inhibition factor (SCIF), liberated by lymphocytes treated with antilymphocytic globulin, causes inhibition of migration of the spleen cells of intact mice. The degree of inhibition of migration corresponds to the colony-inhibiting activity of the SCIF. Macrophage migration inhibition factor (MMIF) obtained in the H-2 system has a stimulating effect on exocolonization in mice on treatment of a bone-marrow graft in vitro. The colony-stimulating activity of MMIF corresponds to its inhibitory effect on migration of spleen cells. Incubation of bone marrow cells with MMIF for 30 min is more effective than incubation for 2 h. It is suggested that SCIF and MMIF are not identical with one another.
KEY WORDS: Stem cell inhibition factor; migration inhibition factor; exocolonization; migration.

Investigation of the biological properties of hematopoietic stem cell inhibition factor (SCIF), which is secreted by lymphocytes treated with antilymphocytic globulin (ALG) [1], has shown that it has the ability to inhibit migration of the spleen cells of intact mice [3].

To study the possible identity of SCIF with marcrophage migration inhibition factor (MMIF), in the investigation described below the action of these factors on hematopoietic stem cells and migration of spleen cells was studied.

EXPERIMENTAL METHOD

SCIF was obtained by the method described earlier [2]. MMIF was obtained in an H-2 system by the method suggested by Friedman [6], in the modification of Suslov et al. [4].

The method of cloning hematopoietic stem cells in vivo in lethally irradiated recipients [10] was used. The bone marrow cells intended for transplantation were treated in vitro with SCIF or MMIF for 120 or 30 min, after which they were washed three times with medium. An intravenous injection of $0.5 \cdot 10^5$ cells of intact or treated bone marrow was given to the recipients 4 h after irradiation (830 rad). The animals were killed nine days later and the number of macroscopically visible colonies on the surface of the spleen was counted.

To test the activity of the resulting MMIF and to study the biological properties of the SCIF, the capillary method of macrophage migration was used [5]. Cells from the intact spleen of (GBA × C57BL/6) F_1 mice were used as migrating cells. The degree of migration was assessed by weighing the zone of migration, traced on

Laboratory of Cellular Immunity, Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR P. A. Vershilova.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsniy, Vol. 86, No. 9. pp. 344-347, September, 1978. Original article submitted April 7, 1978.

TABLE 1. Effect of Different Samples of SCIF and MMIF on Hematopoietic Stem Cells

No. of experiment	Reagent	Number of animals	No. of colonies	Clone formation, %
1	SCIF	9	3,2±0,6	11
	NRG	10	29,1±1,0	100
2	SCIF	13	4,8±0,6	16
	NRG	12	28,3±0,2	100
3	SCIF	15	8,2±0,7	21
	NRG	11	29,7±0,7	100
	MMF	13	11,0±2,0	212
1	LMIF	7	5,0±2,0	100
	Medium No. 199	11	5,3±0,7	
	MMIF	9	13,7±1,0	159
2	LMIF	6	8,6±1,6	100
	Medium No. 199	9	8,2±0,8	
	MMIF	10	12,7±1,8	120
3	LMIF	8	10,6±1,4	100
	Medium No. 199	8	11,8±0,9	
	Exocolonization	10	0	

TABLE 2. Action of Different Samples of SCIF and MMIF on Migration of Spleen Cells

No. of experiment	Materials studied	Degree of migration (M ± m)	Percent inhibition	P
1	SCIF	26,7±1,3	74	0,01
	NRG	76,3±1,7	—	
2	SCIF	28,2±0,4	65	0,01
	NRG	80,5±0,7	—	
3	SCIF	32,5±1,2	59	0,01
	NRG	79,2±0,8	—	
1	MMIF	21,8±2,2	69	0,01
	LMIF	70,4±1,3	—	
2	MMIF	37,9±0,7	42	0,01
	LMIF	65,3±1,7	—	
3	MMIF	48,0±0,5	29	0,01
	LMIF	67,7±0,9	—	

standard paper. By the formula $\frac{P_E - P_C}{P_C} \cdot 100\%$ the percentage inhibition of migration was determined (P denotes the weight of the region of migration, E the experiment, and C the control).

EXPERIMENTAL RESULTS

Several supernatants containing MMIF, which differed in their ability to inhibit migration of spleen cells, and several samples of SCIF, with different colony-inhibiting activity, were used. The results of experiments to study the action of SCIF and MMIF on clone formation are given in Table 1, and the effect of the corresponding samples of the factors on migration is shown in Table 2. It will be clear from these results that SCIF with maximal inhibitory activity against hematopoietic stem cells showed the greatest ability to inhibit migration, of spleen cells (clone formation 11%), whereas the factor inhibiting migration by a lesser degree (59%) possessed weaker colony-inhibiting activity (21%).

A study of the properties of MMIF showed that, unlike SCIF, this factor stimulates colony formation in the spleen of lethally irradiated recipient mice. This property of MMIF corresponded to its ability to inhibit migration of spleen cells. The factor inhibiting migration by 69% was found to have the strongest activating action on transplantable stem cells (clone formation 212%). In the case of inhibition of migration by 42%, clone formation amounted to 159%. Finally, during inhibition of migration by 29%, clone formation was reduced to 119%. The

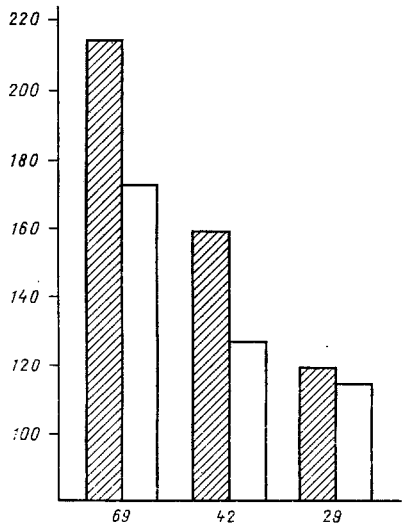

Fig. 1. Dependence of intensity of clone formation on duration of incubation of transplanted bone marrow cells with MMIF. Shaded columns denote incubation of bone marrow cells with MMIF for 30 min; unshaded columns incubation of bone marrow cells with MMIF for 2 h. Each pair of columns represents one sample of the factor. Abscissa, inhibition of migration of spleen cells by different samples of MMIF (in %); ordinate, clone formation (in %).

data given above reflect the results of experiments in which the duration of incubation of the transplantable bone marrow was 30 min. This time was long enough for manifestation of the inhibitory action of SCIF on the stem cells. However, the strongest inhibition was reached after incubation of the bone marrow cells with SCIF for 2 h, and for that reason when the effect of MMIF on the stem cells was studied it was decided to continue the incubation in some of the experiments for 2 h. The results showed (Fig. 1) that during longer incubation of the bone marrow cells with MMIF stimulation of clone formation was preserved, although it became weaker. In this case, therefore, activity of MMIF toward stem cells correlated with the degree of inhibition of migration.

Supernatants used as the control, irrespective of the incubation time, did not affect clone formation.

The conditions of obtaining the humoral factors of cellular immunity were largely similar. Accordingly, to detect new properties of the lymphokines, it was necessary to discover whether this property is characteristic of a given mediator of cellular immunity. The inhibitory action of SCIF on migration of spleen cells could be explained by the identity of SCIF and MMIF, or by the presence of both factors. It was all the more important to shed light on this matter because of recently published investigations in which MMIF was obtained with the aid of mitogens and ALG, in particular, in [8, 9]. The conditions of obtaining SCIF and MMIF, when secreted by intact cells under the influence of mitogens, are thus identical. In the present investigation the MMIF which was used was obtained in the H-2 system, i.e., it was an antigen-dependent factor. However, data indicating the identity of MMIF secreted by sensitized and intact lymphocytes have recently been published [7].

It was shown in that investigation that a supernatant containing MMIF, by contrast with SCIF, possesses stimulating and not inhibitory activity. The degree of stimulation of clone formation corresponded to the intensity of inhibition of migration. On that basis it might be supposed that the stimulating action was the property of the MMIF itself. The stimulation effect could be connected with intensification of recruiting of stem cells and an increase in cloning efficiency. The stronger stimulation observed during incubation of the bone marrow cells with MMIF for 30 min than for 2 h may be due to the fact that, simultaneously with the increase in recruiting, maturation of the stem cells also takes place under the influence of MMIF. As a result, the cells cease to be detected during cloning in lethally irradiated recipients. It can tentatively be suggested that during

incubation for 30 min the recruiting process predominates, and as a result marked stimulation of clone formation takes place. After treatment of the bone marrow cells with MMIF for 2 h, maturation of the stem cells takes place more intensively and the observed stimulation of clone formation decreases. It may also be that as a result of longer incubation redifferentiation of the stem cells takes place with predominance of myelopoiesis and a decrease in the number of foci which can be counted on the surface of the spleen. Ability to inhibit migration of spleen cells is thus one of the properties of SCIF. MMIF and SCIF have different biological properties and they are evidently not identical with one another. The mechanism of the colony-stimulating action of MMIF has not yet been finally explained and requires further study.

LITERATURE CITED

1. D. R. Kaulen, T. A. Golovanova, D. P. Pyatykhina, et al., Byull. Éksp. Biol. Med., No. 2, 64 (1974).
2. D. R. Kaulen, T. A. Golavanova, D. P. Pyatykhina, Folia Biol. (Prague), 21, 95 (1975).
3. S. A. Rigvava, E. B. Mysyakin, D. P. Pyatykhina, et al., in: Early Manifestations of Tissue Incompatibility [in Russian], Moscow (1976), pp. 149-150.
4. A. P. Suslov, S. G. Egorova, and B. D. Brondz, Byull. Éksp. Biol. Med., No. 10, 86 (1975).
5. J. R. David, S. Al-Askari, H. S. Lawrence, et al., J. Immunol., 93, 264 (1964).
6. H. Friedman, Transplantation, 11, 288 (1971).
7. K. Haveman, M. Horvat, C. P. Sodomann, et al., Europ. J. Immunol., 2, 97 (1972).
8. J. R. Lametin and P. Vassali, Nature, 229, 426 (1971).
9. E. Pick, L. Brosloff, L. Krejci, et al., Cell Immunol., 1, 92 (1970).
10. S. Till, and E. McCulloch, Radiat. Res., 14, 213 (1961).