ROLE OF STRAIN DIFFERENCES IN PRODUCTION OF STEM CELL INHIBITION FACTOR

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UDC 612.017.1-06:612.6.05

KEY WORDS: mediators of cellular immunity (lymphokines); hematopoietic stem cell; stem cell inhibition factor; chief histocompatibility complex; genetic control.

The genetic determination of immune reactions has now been firmly proved and the concrete genes of the immune response (Ir-genes) have been discovered and characterized [7, 9, 11]. However, genetic control of lymphokine production has so far received little study although isolated investigations have shown that the synthesis of some mediators of cellular immunity is under the control of the chief histocompatibility complex [4, 6, 11]. The role of strain differences in lymphokine (interleukin) induction has not yet been fully explained and the data are highly contradictory. Strains of mice differing in their ability to produce macrophage migration inhibition factor (MIF) [6, 17] and interferon [10] have been discovered experimentally, whereas no strain differences have been found in induction of colony-stimulating factor (CSF) [13]. Since in its physicochemical properties a lymphokine described by the present writers — stem cell inhibition factor (SCIF) — more closely resembles mediators of MIF and interferon type, with a well developed spectrum of action [1, 2, 5], it was decided to study the role of strain differences in its liberation.

EXPERIMENTAL METHOD

Experiments were carried out on mice of strains CBA, BALB/c, DBA/2, C57BL, CC57Br, AKR, and A/Sn and on (CBA × C57BL)F_1 hybrids. Antilymphocytic globulin (ALG) was isolated from antilymphocytic serum, obtained by immunization of rabbits with thymus cells from hybrid mice [3]. To obtain SCIF, intact thymus cells (30×10^6 cells/ml) were treated *in vitro* with ALG in a concentration of 5 mg/ml for 30 min at 37°C, after which the cells were washed 3 times in a large volume of medium 199 and then incubated in "pure" MEM medium for 2 h at 37°C. The resulting supernatant, containing SCIF, was tested for its ability to inhibit colony formation induced by bone marrow cells in an exocolonization system [16]. Supernatant from thymocytes treated in the same way with normal rabbit globulin served as the control. Immunochemical analysis excluded the presence of ALG, eliminated from the surface of the SCIF-producing cells, in the supernatants [8]. To study the role of strain differences in liberation of SCIF humoral factors were obtained by the method described above, using thymocytes from mice of different haplotypes as its traducers. The test system was chosen by the method of cloning hematopoietic cells *in vitro* [16]. Bone marrow cells intended for transplantation ($1 \cdot 10^7$ cells/ml) were treated *in vitro* with different specimens of factors for 1 h at 37°C. After incubation the cells were washed with medium 199, adjusted to the necessary concentration, and injected into lethally irradiated (830 rads) recipients. On the 9th day after transplantation and irradiation the animals were killed, the spleens were removed, and the number of macroscopically visible colonies on the surface of the organ counted after fixation. The significance of differences between mean values was determined by Student's t test at the $P < 0.01$ level.

EXPERIMENTAL RESULTS

The results of the study of the effect of humoral lymphocytic factors on thymocytes of different inbred strains of mice by the technique described above are summarized in Table 1.

Laboratory of Cellular Immunity, N. F. Gamaleya 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 Meditsiny, Vol. 93, No. 6, pp. 103-106, June, 1982. Original article submitted December 24, 1981.

0007-4888/82/9306-0823$07.50

TABLE 1. Comparative Analysis of Action of Lymphocytic Humoral Factors Liberated by Activated Thymocytes from Mice of Different Haplotypes on Hematopoietic Stem Cells

Group No.	Reagent	Haplotype of thymocytes producing humoral lymphocytic factor	Number of animals tested	Number of hematopoietic colonies per spleen (M ± m)	Percent inhibition of clone formation
1	Factor I	C57BL ($H\text{-}2^b$)	9	1,3 ± 0,36	85,7
	CS	"	11	9,1 ± 1,27	—
2	Factor II	A/Sn ($H\text{-}2^a$)	18	1,1 ± 0,22	90,4
	CS	"	13	11,4 ± 0,84	—
3	Factor III	CBA ($H\text{-}2^k$)	7	2,1 ± 0,54	80,0
	CS	"	8	10,5 ± 0,83	—
4	Factor IV	BALb/c ($H\text{-}2^d$)	13	1,7 ± 0,42	82,5
	CS	"	9	9,7 ± 0,48	—
5	Factor V	DBA/2c ($H\text{-}2^d$)	10	2,4 ± 0,71	80,5
	CS	"	7	12,3 ± 1,12	—
6	SCIF	F_1 (CBA×C57BL) ($H\text{-}2^k \times H\text{-}2^b$)	16	1,4 ± 0,46	84,4
	CS	"	17	9,0 ± 1,02	—
7	Medium 199	—	10	10,1 ± 0,90	—
8	Irradiation control		17	—	

TABLE 2. Effect of SCIF on Bone Marrow Cells from Mice of Different Haplotypes

Group No	Reagent used to treat target cells	Type of transplantation (donor of cells → recipient)	Number of cells transplanted, $\times 10^5$	Haplotype of target cells	Number of animals studied	Number of hematopoietic colonies per spleen (M ± m)	Per cent inhibition of clone formation
1	SCIF	A/Sn→F_1	15	A/Sn ($H\text{-}2^a$)	10	1,5±0,50	85,5
	CS	"	15	"	11	10,8±1,40	—
	Medium 199	"	15	"	8	10,5±1,22	—
2	SCIF	AKR→F_1	15	AKR ($H\text{-}2^k$)	10	1,5±0,50	90,6
	Medium 199	"	15	"	9	16,4±1,13	—
	CS	"	15	"	8	16,0±1,18	—
3	SCIF	BAL b/c→F_1	5	BALb/c ($H\text{-}2^d$)	12	2,1±0,87	83,7
	CS	"	5	"	10	13,5±1,15	—
	Medium 199	"	5	"	11	12,1±0,97	—
4	SCIF	CC57Br→F_1	10	CC57Br ($H\text{-}2^b$)	9	0,2±0,22	95,8
	CS	"	10	"	8	5,4±0,84	—
	Medium 199	"	10	"	7	5,3±1,01	—
5	SCIF	F_1→F_1	1	F_1($H\text{-}2^k \times H\text{-}2^b$)	12	1,4±0,46	84,6
	CS	"	1	"	9	9,4±0,98	—
	Medium 199	"	1	"	13	10,1±0,98	—
6	Irradiation control				17	0,1±0,04	

Legend. F_1) First generation (CBA × C57BL) hybrids.

Transplantation of bone marrow cells treated *in vitro* with medium 199 or the control supernatant (SC) led to the formation of 10.1 and 9.0 hematopoietic colonies respectively in the spleens of the lethally irradiated recipients (Table 1). Meanwhile similar treatment of transplantable syngeneic bone marrow cells with SCIF caused a statistically significant decrease in colony formation, and on average 1.4 foci were counted in the spleens of the experimental mice (Table 1). The H-2 system was found to exhibit an unusually high degree of polymorphism and, like the majority of the loci studied, it has multiple alleles [9]. With this in mind, when obtaining humoral lymphocytic factors thymocytes from mice of standard strains with different haplotypes ($H\text{-}2^a$ and $H\text{-}2^b$, differing in specificities 2, 3, 4, 8, 11, and 33, and also thymocytes with haplotypes $H\text{-}2^b$ and $H\text{-}2^d$ differing in specificities 2, 3, 4, 5, 9, 31, and 39) were used. Supernatants obtained from activated thymocytes from CBA, C57BL, A/Sn, BALB/c, and DBA/2 mice, with different haplotypes, contained activity directed against hematopoietic stem cells (HSC), for they all induced marked inhibition of clone formation and, in their inhibitory activity, they were practically equal to SCIF (Table 1).

Thymocytes from mice of different haplotypes, activated by ALG *in vitro*, can thus liberate activity into the culture medium similar to that of SCIF, with a powerful inhibitory action on HSC, irrespective of whether syngeneic, semisyngeneic, or allogeneic thymocytes

relative to the bone marrow cells forming exogenous hematopoietic colonies in spleens of the lethally irradiated recipients were used as producers of the factor.

In another series of experiments the effect of SCIF was studied on transplantable bone marrow cells from mice of different haplotypes. SCIF were obtained as usually on thymocytes of F_1 hybrids. Mice of different strains are known to differ in their resistance to exposure to ionizing radiation, and hybrids have the greatest resistance to irradiation. Accordingly (CBA × C57BL)F_1 hybrids were used as recipients of the transplanted bone marrow cells in this series of experiments (just as in the previous one). However, investigation of HSC function in lethally irradiated recipients showed that after injection of allogeneic bone marrow cells the number of exogenous colonies observed in the spleen was much smaller than after injection of syngeneic cells. This phenomenon has been called allogeneic inhibition or genetic hematopoietic resistance. It has also been shown that allogeneic inhibition can be avoided by the use of several special methods. Genetic resistance to a bone marrow graph can be largely overcome by transplantation of a larger dose of cells than is usual in a syngeneic microenvironment into nonsyngeneic irradiated recipients. Accordingly, for all strains of mice used in the investigation a definite dose of bone marrow cells was chosen which would not lead to the formation of a truly countable number of hematopoietic colonies (Table 2). On treatment of transplanted bone marrow cells from mice of different haploid types with SCIF *in vitro* a statistically significant decrease was observed in the number of hematopoietic foci in all groups tested. Comparison of the ability of SCIF to inhibit exocolonization did not reveal any strain of mice whose bone marrow cells were resistant to its action, probably because of the absence of genetic restriction of the suppressor activity of SCIF, for the reduction in clone formation was considerable in all groups and amounted to between 84 and 95%.

Usually immunologic reactions effected in mice chiefly at the T cell level are controlled by genes linked with the H-2 complex of the chief histocompatibility locus. Genetic control over the production of certain lymphokines may perhaps be effected not at the level of the complete H-2 system, but restricted to particular subregions of the H-2 locus. For instance, a clear effect of genotype on MIF production has been established [6, 17], whereas the factors produced by T cells and binding immunoglobulin (IBF) carries determinants coded by region I of the chief histocompatibility complex, and it does not exhibit allogeneic restriction [14], like SCIF. On the basis of the results described above it can confidently be stated that SCIF does not possess strain specificity, for these experiments revealed no strain differences in respect of the production of this lymphokine.

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