INACTIVATION OF NONSYNGENEIC PROCURSOR CELLS INDUCING A GRAFT VERSUS HOST REACTION IN MICE

É. I. Panteleev and R. M. Khaitov

UDC 612.6.02.017.1

In experiments on (CBAT6T6 × C57B1) F_1 mice 25×10^6 spleen cells were transplanted from CBAT6T6 or C57B1 donors or a mixture of the same number of spleen cells of the two parental genotypes was transplanted. Combined transplantation of lymphoid cells of the 2 parental genotypes into F_1 recipients was accompanied by their interaction, leading to considerable or complete inhibition of proliferation of cells of the C57B1 genotype. As a result, the graft versus host reaction in the group of recipients receiving the mixture of cells was much less marked than when the cells of these genotypes were transplanted separately.

A method of quantitative analysis of the interaction between stem and lymphoid cells was developed previously [2] and it was shown that transplantation of a mixture of hematopoietic or lymphoid cells from mice of two different genotypes into lethally irradiated recipients is accompanied by inactivation of hematopoietic colony-forming units (CFUs) of the graft. Experiments have shown that not only CFUs, but also precursor cells of antibody-forming cells also are inactivated [6]. Later work showed that during interaction between cell populations of the two different genotypes in lethally irradiated recipients stem cells are inactivated not only in the spleen, but also in the bone marrow, lymph glands, and thymus, i.e., in places with a different character of differentiation [3, 7]. Inactivation of nonsyngeneic osteoblasts by lymphocytes has been demonstrated [4].

The object of the present investigation was to study interaction between lymphoid populations of 2 parental genotypes in normal, unirradiated F_1 recipients in order to discover whether inactivation of precursor cells of the immunocytes responsible for the graft versus host reaction takes place. Two methods were used: a model of the graft versus host reaction (GVHR) and cytogenetic analysis of the origin of the proliferating cells in the hematopoietic and lymphoid tissues of the recipient.

EXPERIMENTAL METHOD

Inbred mice of lines CBAT6T6 and C57B1 and (CBAT6T6 × C57B1) F_1 hybrids were used in the experiments. Intact (CBAT6T6 × C57B1) F_1 mice aged 1.5-2 months and weighing 14-16 g were used as recipients. Animals of parental genotypes of the same sex (♂) and aged 3-4 months were used as donors of spleen cells. The recipient mice were injected with 25×10^6 CBAT6T6 or C57B1 spleen cells or a mixture of 12.5×10^6 cells of each genotype. Control animals of the same weight and age were not injected with cells. The injection of syngeneic spleen cells is known not to induce a GVHR [1]. Preliminary experiments showed that the GVHR reached its maximal intensity 2 weeks after transplantation of the parental cells. One feature of the development of the GVHR is a sharp increase in the size and weight of the spleen (splenomegaly) in the recipients. To detect the GVHR by means of this criterion the recipient mice were sacrificed on the 14th day after transplantation and their splenic index was determined by Simonsen's method [9]. The relative weight of the spleen (RWS) was first determined as the ratio between the weight of the spleen (in milligrams) and the body weight (in grams). The relative weight of the spleen of the dif-

(Presented by Academician of the Academy of Medical Sciences of the USSR P. D. Gorizontov.)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 73, No. 4, pp. 86-89, April, 1972. Original article submitted July 2, 1971.

TABLE 1. Appearance of GVHR After Transplantation of Mixture of Lymphoid Cells of Parental Genotypes into F_1 Hybrid Recipient

Donor of spleen cells (25×10^6)	No. of mice	Expt. № 1		No. of mice	Expt. № 2	
		RWS	SI		RWS	SI
CBA	20	8,94±0,39 (8,12÷9,76)	1,60	5	12,75±0,71 (10,78÷14,72)	1,86
C57Bl	20	10,35±0,25 (9,83±10,87)	1,85	10	10,63±0,68 (9,04÷12,17)	1,55
Mixture CBA + C57Bl	20	7,33±0,26 (6,79÷7,87)	1,31	13	7,46±0,36 (6,68÷8,24)	1,10
Control	10	5,58±0,15 (5,25÷5,91)	1,00	10	6,85±0,15 (6,51÷7,19)	1,00

Note. Confidence interval with probability 95% shown in parentheses.

TABLE 2. Cytological Identification of Dividing Cells in Spleen of (CBAT6T6 × C57Bl) F_1 Recipients After Transplantation of Spleen Cells of Parental Genotypes

Time after transplantation (in days)	Genotype of cells injected	Number of mitoses investigated	Genotype of dividing cells					
			CBAT6T6		C57Bl		(CBAT6T6 × C57Bl) F_1	
			abs.	%	abs.	%	abs.	%
7	CBAT6T6	57	3	5,2	—	—	54	94,8
	C57Bl	39	—	—	7	17,9	32	82,1
	Mixture	49	3	6,1	2	4,0	45	89,9
14	CBAT6T6	51	1	1,9	—	—	50	98,1
	C57Bl	60	—	—	8	13,3	52	86,7
	Mixture	58	2	3,4	0	0	56	96,6
30	CBAT6T6	43	4	9,3	—	—	39	90,7
	C57Bl	70	—	—	67	95,6	3	4,4
	Mixture	30	0	0	10	33,3	20	66,7
60	CBAT6T6	57	0	0	—	—	57	100,0
	C57Bl	43	—	—	43	100,0	0	0
	Mixture	45	0	0	0	0	45	100,0

ferent groups was then used to determine the splenic index (SI) by dividing the RWS of the experimental group by the RWS of the control group. In these experiments the ratio between the maximal individual value of the RWS and the minimal value in the control series never exceeded 1.27. In the experimental groups of animals, a value of SI greater than 1.27 was regarded as evidence of the development of a GVHR in that group. The higher the value of SI in the experimental group, the more marked the intensity of the GVHR. Chromosome analysis of the recipient's spleen and bone marrow was carried out by Ford's method 7-60 days after transplantation.

EXPERIMENTAL RESULTS

In the group in which the recipients received a mixture of parental spleen cells the value of SI was 1.31 in experiment No. 1 and 1.10 in experiment No. 2, evidence of very weak development of the GVHR. However, the same number of transplanted cells of any one parent genotype induced a much stronger GVHR. After transplantation of CBAT6T6 spleen cells the SI in two different experiments was 1.60 and 1.86, while after transplantation of C57Bl cells its value was 1.85 and 1.55 respectively. The intensity of the GVHR was thus reduced after transplantation of a mixture of lymphoid cells of two foreign genotypes by comparison with that observed after their transplantation separately.

The percentage of dividing cells in the recipients' spleen after combined or separate transplantation of spleen cells of two genotypes is illustrated in Table 2. After transplantation of CBAT6T6 cells, as a

TABLE 3. Cytological Identification of Dividing Cells in Bone Marrow of (CBAT6T6 × C57B1) F_1 Recipient After Transplantation of Spleen Cells of Parental Genotype

Time after transplantation (in days)	Genotype of cells injected	Number of mitoses investigated	Genotype of dividing cells					
			CBAT6T6		C57B1		(CBAT6T6 ×C57B1) F_1	
			abs.	%	abs.	%	abs.	%
7	CBAT6T6	50	1	2	—	—	49	98,0
	C57B1	40	—	—	3	7,5	37	92,5
	Mixture	55	4	7,2	1	1,8	50	91,0
14	CBAT6T6	72	3	4,1	—	—	69	95,9
	C57B1	68	—	—	6	8,8	62	91,2
	Mixture	64	2	3,1	1	1,5	61	95,9
30	CBAT6T6	31	2	6,4	—	—	29	93,6
	C57B1	16	—	—	16	100,0	0	0
	Mixture	17	1	5,8	2	11,6	14	82,6
	CBAT6T6	93	0	0	—	—	93	100,0
	C57B1	75	—	—	75	100,0	0	0
	Mixture	114	0	0	0	0	114	100,0

rule the proliferating transplanted cells constituted the smallest fraction of the dividing spleen cells from the 7th until the 30th day after injection (5.2-9.3%). Two months after transplantation no dividing cells of the CBA genotype could be detected in the recipient's spleen. Cells of genotype C57B1 proliferated quite differently in the recipient's spleen. On the 7th-14th day after injection 13-18% of the dividing cells were donor's in origin. After 30 days 95% of mitoses were in donor's cells, and after 60 days the number had risen to 100%. Transplantation of a mixture of spleen cells of these two genotypes was accompanied by a sharp decrease in the percentage of proliferating cells of the C57B1 genotype. On the 7th day after injection of the mixture, for instance, 6.1% of cells of the CBA genotype was found in the recipient's spleen, while the number of mitoses of the C57B1 genotype was reduced to 4% (18% in the case of separate transplantation). No dividing C57B1 cells could be found 14 days after transplantation of a mixture, whereas there were 13% after separate transplantation; at this time the number of dividing cells of the CBA genotype was 3.4%. On the 30th day after injection of the mixture the percentage of C57B1 mitoses was reduced to 33 compared with 95 after separate transplantation. All the dividing cells were of the recipient genotype 60 days after transplantation.

It is clear from Table 3, which gives the results of chromosome analysis of the bone marrow of this group of recipients that the picture of chimerism in the bone marrow reproduced in its basic features the dynamics of repopulation of the recipients' spleen by injected donors' cells in the various experimental groups.

The results showing the development and expression of the GVHR in the various experimental groups of mice, together with the results of chromosome analysis, thus indicate that combined transplantation of lymphoid cells of both parental genotypes into a normal F_1 recipient is also accompanied by their interaction, leading to marked or complete inhibition of proliferation of the C57B1 cells. As a result, the intensity of the GVHR in the group of recipients receiving a mixture of cells was much less than after transplantation of cells belonging to only one parental genotype. In that case, just as in the phenomenon of inactivation of nonsygeneic stem cells [7], the cells of the C57B1 genotype were evidently "targets," and were inactivated in the recipient's body by lymphocytes of the CBA genotype.

Inactivation of nonsyngeneic hematopoietic stem cells or precursors of antibody-forming cells is known to take place as the result of the direct action of lymphocytes of one genotype on stem target cells of another genotype [4, 7]. It is also known, however, that a GVHR can be induced by transplantation of purified lymphocytes [5, 8] capable of proliferating in the recipient's body. Lymphocytes of the C57B1 genotype, capable of inducing a GVHR, are also suppressed by the CBA lymphocytes, just as in the phenomenon of inactivation of nonsyngeneic stem or antibody-forming cells.

In the light of these observations it is logical to conclude that not all of the lymphocyte population are initiators of the GVHR, but only some of them. The lymphocytes inducing this reaction, like stem cells, must be along the road to differentiation and proliferation.

LITERATURE CITED

1. I. N. Golovistikov, Postradiation Transplantation of Hematopoietic Tissues from Tolerant and Sensitized Donors. Author's Abstract of Candidate's Dissertation, Moscow (1967).
2. R. V. Petrov and L. S. Seslavina, Dokl. Akad. Nauk SSSR, 176, No. 6, 1170 (1967).
3. R. M. Khaitov, Byull. Éksperim. Biol. i Med., No. 6, 88 (1970).
4. V. N. Shvets and V. M. Man'ko, Byull. Éksperim. Biol. i Med., No. 6, 72 (1970).
5. J. Gowans, Ann. New York Acad. Sci., 99, 432 (1962).
6. R. V. Petrov, L. S. Seslavina, and E. J. Panteljev, Nature, 217, 558 (1968).
7. R. V. Petrov, R. M. Khaitov, and V. S. Yegorova, Folia Biol. (Prague), 16, 29 (1970).
8. K. Porter, C. Chapius, and M. Freeman, Ann. New York Acad. Sci., 99, 663 (1962).
9. M. Simonsen and E. Jensen, in: Biological Problems of Grafting, Springfield (1959), p. 1.