

# QUANTITATIVE EVALUATION OF THE GRAFT-VERSUS-HOST SYNDROME

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Various numbers of lymph gland cells of the parental CBA genotype were transplanted into sublethally irradiated (500-700 R) (CBA  $\times$  C57BL) $F_1$  mice. The allogeneic lymphocytes suppressed colony formation in the spleen of the sublethally irradiated recipients on account of inactivation of the endogenous stem cells (CFU). The degree of suppression of endogenous CFU correlates directly with the number of injected lymphocytes and with mortality among the recipients from the graft versus host reaction. The degree of inhibition of endogenous colony formation after transplantation of lymphocytes of the parental genotype into sublethally irradiated  $F_1$  recipients can provide a quantitative measure of the graft-versus-host syndrome.

A previous investigation [2] showed that lymph gland cells, when transplanted into sublethally irradiated hybrid recipients, suppressed colony formation in the spleen on account of inactivation of endogenous stem cells (CFU). The degree of suppression of the endogenous CFU is directly dependent on the number of lymphocytes injected ( $r=0.93-0.97$ ). This system was used to develop an experimental model to allow quantitative comparison of the action of immunodepressants in 1 experiment with reference to their two principal criteria: their mitostatic and lymphocytotoxic action [3-6].

Determination of the correlation between the degree of inhibition of endogenous CFU and the severity of the graft versus host reaction is of great importance to future research. If such a correlation exists, depression of endogenous colony formation can be used as a quantitative measure of the severity of the graft-versus-host reaction.

The results of experiments to investigate this problem are described below.

## EXPERIMENTAL METHOD

Mice of inbred line CBA and (CBA  $\times$  C57BL) $F_1$  hybrids aged 3-4 months were used in the experiments. The animals were irradiated with  $Co^{60}$   $\gamma$ -rays on a type EGO-2 apparatus in doses of 500, 600, and 700 R. The lymph gland cells were transplanted intravenously 2-4 or 24 h after irradiation. The decrease in the number of endogenous splenic colonies under the influence of the transplanted lymphocytes was expressed as a percentage of the number of endogenous colonies in the spleen of the control animals. To establish the correlation between the acute mortality (graft-versus-host syndrome) and suppression of endogenous CFU of the recipients receiving the grafts, the coefficient of correlation ( $r$ ) was calculated [1].

## EXPERIMENTAL RESULTS

The results demonstrating correlation between the degree of suppression of endogenous colony formation with the severity of the graft-versus-host syndrome, tested with reference to mortality among the recipients, under the influence of transplanted allogeneic lymphocytes are summarized in Table 1. Clearly the transplantation of  $0.2 \times 10^6$  lymph gland cells of the CBA genotype into sublethally (600 R) irradiated (CBA  $\times$  C57BL) $F_1$  recipients did not suppress the multiplication of endogenous CFU and was not accompanied by

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TABLE 1. Correlation between Inhibition of Endogenous CFU and Mortality among (CBA × C57BL)F<sub>1</sub> Mice Irradiated in a Dose of 600 R after Transplantation of Lymphocytes of the CBA Genotype

No. of cells transplanted (× 10 <sup>6</sup> )	No. of animals	No. of endogenous colonies per spleen (M ± SE)	Inhibition of endo-thelial CFU (in %) CFU (in %)	30-day mortality (in %)	Degree of correlation between mortality among recipients and inhibition of CFU
—	40	11,8±0,8 (20)	—	0	r=0,96, level of probability 99%
0,2	40	11,6±0,6 (20)	1,7	5	
0,4	40	7,0±0,8 (18)	40,7	25	
0,6	40	3,2±0,5 (16)	72,9	45	
0,8	40	1,0±0,1 (17)	91,6	96	
1,0	40	0,2±0,1 (15)	98,4	90	

Note. Each group consisted of 40 mice, and 20 of them were left for analysis of the survival rate; the mice of the other half of each group were sacrificed on the 10th day for colonies to be counted (number of spleens examined shown in parentheses).

TABLE 2. Correlation between Inhibition of Endogenous CFU and Mortality among (CBA × C57BL)F<sub>1</sub> Mice Irradiated in Sublethal Doses after Transplantation of Lymphocytes of the CBA Genotype

Dose of irradiation (in R)	No. of cells transplanted (× 10 <sup>6</sup> )	No. of animals	Number of endogenous colonies per spleen (M ± SE)	Inhibition of endo-thelial CFU (in percent)	10-day mortality (in %)	Degree of correlation between mortality among recipients and inhibition of CFU
700	—	40	4,7±0,8 (20)	—	0	r=0,8, level of probability 99%
	0,1	20	4,6±1,0 (10)	2,2	0	
	0,2	34	2,1±0,7 (14)	55,4	30	
	0,4	33	1,2±0,8 (13)	74,5	35	
	0,8	32	0,4±0,2 (10)	91,5	54,5	
500	—	40	21,2±1,2 (20)	—	0	r=0,9, level of probability 99%
	0,2	17	17,1±2,2 (7)	18,6	30	
	0,4	17	13,8±2,4 (7)	34,3	30	
	0,8	32	7,9±1,3 (12)	62,4	40	
	1,6	32	1,1±0,4 (12)	94,8	40	
	2,4	32	0,8±0,3 (10)	96,2	54,5	
	3,2	36	0,1±0,09 (10)	99,6	61,6	

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Note. Number of spleens examined given in parentheses.

mortality of the recipients during the 30 days after irradiation. A dose of  $0.4 \times 10^6$  CBA lymphocytes inhibited 40.7% of endogenous CFU, and led to the death of 25% of the recipients. Transplantation of  $0.6 \times 10^6$  allogeneic lymphocytes suppressed 72.9% of the CFU and was accompanied by death of 45% of the animals. Transplantation of  $0.8-1.0 \times 10^6$  allogeneic lymphocytes led to 90-100% inhibition of endogenous CFU and to maximal mortality (90-95%) of the recipients during the 30-day period. Statistical analysis showed a strong direct correlation ( $r=0.96$ ; level of probability 99%) between mortality among the recipients and inhibition of endogenous CFU.

In the experiments described above the different number of lymphocytes were transplanted into recipients irradiated in a dose of 600 R. In the next series of experiments not only the dose of lymphocytes transplanted, but also the dose of irradiation of the recipient varied.

The mortality rates among the sublethally irradiated (500 and 700 R) (CBA × C57BL)F<sub>1</sub> recipients receiving different doses of lymphocytes of the parental CBA genotype, are given in Table 2 and compared with inhibition of colony formation in their spleens. As Table 2 shows, in these cases also strong direct correlation ( $r=0.8$  and  $0.9$ ; level of probability 99%) also was obtained between inhibition of endogenous colony formation in the sublethally irradiated F<sub>1</sub> recipients and the intensity of the graft-versus-host reaction.

The results thus demonstrate that the degree of inhibition of endogenous colony formation after transplantation of lymphocytes of the parental genotype into sublethally irradiated F<sub>1</sub> recipients can be used as an accurate quantitative index of the graft-versus-host syndrome.

#### LITERATURE CITED

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