

EXPERIMENTAL BIOLOGY

INACTIVATION OF COLONY-FORMING ACTIVITY OF EMBRYONIC HEMATOPOIETIC CELLS IN TRANSPLANTATION FROM TWO GENETICALLY INCOMPATIBLE DONORS

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Lethally irradiated F_1 (C57B1 \times CBA) hybrid mice were injected with $1 \cdot 10^6$ – $3 \cdot 10^6$ embryonic mouse liver cells from parent lines separately or in combination. After combined transplantation the number of spleen colonies was reduced by comparison with the total number of colonies formed when cells of each line were injected separately. The index of inactivation of the number of colony-forming units varied in the different experiments from 14 to 69%.

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This paper gives the results of a further study of the phenomenon, previously discovered by Petrov and Seslavina [1], of inactivation of stem cells after the combined transplantation of two genetically different cell populations.

Transplantation of hematopoietic tissue cells into irradiated mice leads to the formation of hematopoietic colonies in the spleen [4]. Petrov and Seslavina showed that if cells from more than one donor are mixed, and the inoculum consists of cells from mice of two different lines, fewer colonies appear than after transplantation of the same cell suspensions into different recipients separately. The nature of this inhibition of colonization and its place among the other transplantation reactions are not yet fully clear. Among the cell reactions to nonsyngenic material, besides examples of the response of immunologically competent cells already known, phenomena have been described in which immunologically incompetent cells were the reacting elements. These phenomena include the blast transformation of immunologically incompetent lymphocytes in the newborn and the acceleration of growth of a culture of lymphoblasts and amnion cells described by Eagle and Levine [2, 3] during their combined cultivation.

Since any new phenomenon is always best compared in a particular situation with the classical concepts in that field, the temptation to carry out a mixed colonization experiment using embryonic donors was very great. Such an experiment could show whether immunologic competence of the cells in the classical interpretation of transplantation immunology was necessary for occurrence of the phenomenon of inhibition of mixed colonization.

EXPERIMENTAL METHOD

As in the preceding investigations, F_1 (C57B1 \times CBA) mouse hybrids aged 3 months, irradiated with γ -rays in a dose of 850 R on the ÉGO-2 apparatus, were used as recipients. The donor material consisted of a suspension of liver cells from 17-day embryos (weight about 0.8 g) of mice of lines C57B1/Sc/Sn and CBA. A dose of $1 \cdot 10^6$ – $3 \cdot 10^6$ cells per mouse, giving from 5 to 10–12 colonies in the spleen, was chosen empirically. Usually from 6 to 8 embryos were taken, and their liver (weight about 0.25 g) was suspended in medium No. 199 and filtered. After centrifugation at 800 rpm in a rotor 26 cm in diameter for 5 min, the residue was resuspended in 10 ml. This suspension contained about $15 \cdot 10^6$ – $30 \cdot 10^6$ cells/ml. The recipients were sacrificed 8 days after the injection, and after fixation in Bouin's solution the colonies were counted. For cytologic analysis of the colonies some mice were sacrificed on the 11th day, the colonies were punctured with a glass needle with a tip 200–300 μ in diameter, compressed with ophthalmic forceps, and the expressed droplets added to a drop of mouse serum on a slide. The drop of serum must not exceed

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TABLE 1. Number of Hematopoietic Colonies after Combined and Separate Transplantation of Embryonic Hematopoietic Cells. Number of Colony-Forming Units (CFU) Calculated per 10^6 Injected Donor Cells

Experiment No.	Donors	Number of recipients	Number of CFU \pm standard error	Expected number of CFU	Index of inactivation = $\frac{(1 - \frac{\text{CFU obtained}}{\text{CFU expected}}) \times 100}{1}$
1	C57Bl	4	9,7 \pm 2,6	10,5	69
	CBA	3	11,2 \pm 3,8		
	mixed	4	3,3 \pm 1,8		
2	C57Bl	4	1,2 \pm 0,2	5,5	51
	CBA	4	9,7 \pm 2,1		
	mixed	2	2,7 \pm 0,7		
3	C57Bl	5	5,5 \pm 1,3	3,8	28
	CBA	3	1,8 \pm 0,6		
	mixed	7	2,5 \pm 0,9		
4	C57Bl	8	1,7 \pm 0,4	5,5	14
	CBA	9	9,8 \pm 0,8		
	mixed	10	4,8 \pm 1,5		
5	C57Bl	5	3,7 \pm 1,6	8,4	36
	CBA	8	13,1 \pm 1,3		
	mixed	5	5,4 \pm 0,5		

1-2 mm³. A film was then made and stained by the Giemsa-Romanovsky method.

EXPERIMENTAL RESULTS

The number of spleen colonies in the case of mixed donors' cells was smaller than would be expected in the case of simple addition of the effects produced by the components of the mixed inoculum (Table 1). On this basis, it must be accepted that colony formation is inhibited by interaction between the components of the mixture. This inhibition of colony development is difficult to explain in terms of the usually accepted concepts of transplantation immunology, for the donor embryos were immunologically incompetent. The immunologic competence of mouse cells develops during postnatal ontogenesis, and the material used for transplantation consisted of hematopoietic liver cells of 17-day mouse embryos. At this stage the embryonic liver functions as a hematopoietic organ. The cells produced in it are erythroid cells (the suspension consists mainly of erythroblasts). The colonies also were erythroid in nature. Myeloid colonies were the exception.

Explanation of the inactivation phenomenon by the influence of the recipient's thymus on the transplanted cells and their rapid maturation is unlikely, for the inocula contained virtually no lymphoid cells. However, no data were obtained which completely reject such an explanation.

If the phenomenon studied in this investigation is not one of the classical phenomena of transplantation immunology, the interaction between nonsyngenic cells must evidently be one of more general type, resembling the cell response mentioned above in the phenomena of blast transformation of nonatal lymphocytes or acceleration of growth of a culture of mixed fibroblasts. It may also be postulated that competition occurs between nonsyngenic series of cells, in addition to the immunologic response, leading to inhibition of the slowly reproducing series.

LITERATURE CITED

1. R. V. Petrov and L. S. Seslavina, Dokl. Akad. Nauk SSSR Seriya Biol., **176**, No. 5, 1170 (1967).
2. H. Eagle and E. M. Levine, Nature, **213**, 1102 (1964).
3. K. Lindahe-Kissling and J. A. Book, Lancet, **2**, 1012 (1964).
4. J. E. Till and E. A. McCulloch, Radiat. Res., **14**, 213 (1961).