"Xenogeneic Resistance" to Rat Bone Marrow Transplantation
I. The Basic Phenomenon[1] (37272)

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In the course of studies to determine the role of the hemopoietic inductive microenvironments (HIM) of the mouse spleen on the differentiation and growth of rat bone marrow stem cells (1), we observed in supralethally irradiated C57BL/6 and (C57BL/6 × A)F_1 hybrid mice, but not in strains A, BALB/c, CBA, DBA, or C3H—a peculiar resistance to rat bone marrow transplants. In preliminary reports we have designated this phenomenon as "xenogeneic resistance" (2, 3). In the present study hemopoietic spleen colony data and karyotype analyses are presented which further document and quantitate this phenomenon.

Materials and Methods. Three- to 9-mo-old female mice of strains A (H-2^a), C57BL/6 (H-2^b), BALB/c (H-2^d), DBA (H-2^d), C3H (H-2^k), CBA (H-2^k), and (C57BL/6 × A)F_1 hybrids (H-2^b/H-2^a) were obtained from the barrier sustained specific pathogen-free (SPF) mouse colony of the Division of Experimental Biology (4). All mouse recipients of xenogeneic bone marrow were supralethally irradiated (950–1100 R, whole body), at an exposure rate of 145 R/min, using a specially constructed ^{137}Cs irradiator with vertically opposed radial sources. These exposures were found to completely suppress endogenous colony formation in spleens of all mouse strains employed.

Inbred female SPF Lewis rats, weighing 150–175 g, were obtained from Charles River Labs, and served as bone marrow donors. Techniques of bone marrow preparation and injection have been previously described (5).

Quantitation of xenogeneic resistance to hemopoietic regeneration was made by means of the spleen colony system, using both the gross and microscopic counting methods (5).

Donor (rat) origin of hemopoietic colonies in both the spleen and bone marrow was verified by a slight modification of the karyotype analysis method already published (6).

Results. Figure 1 shows the mean number of 8-day gross hemopoietic colonies per mouse spleen plotted as a function of increasing rat bone marrow cell dose for 7 strains of mice of 5 different H-2 genotypes. Spleens of strains A, BALB/c, C3H, DBA, and CBA showed grossly countable colonies when mice were injected with doses between 10^5 to 10^6 rat bone marrow cells, and confluent splenic repopulation with doses of 10^6 cells or more. However, spleens of C57BL/6 mice and the (C57BL/6 × A)F_1 hybrid did not show either gross or microscopic hemopoietic regeneration unless much greater rat bone marrow

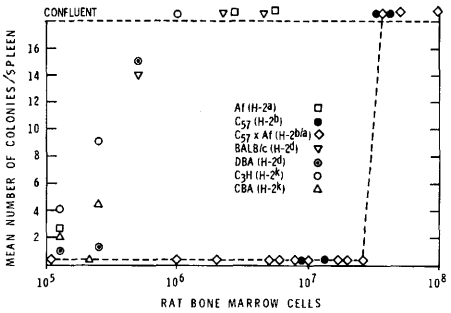

FIG. 1. Xenogeneic spleen colonies (rat to mouse). Eight-day gross spleen colonies in spleens of different mouse strains following supralethal irradiation (950–1100 R) and infusion of graded doses of rat bone marrow cells. Striking resistance to hemopoietic repopulation by rat bone marrow is seen in irradiated C57BL/6 mice and their F_1 hybrids (C57BL/6 × A), but not in strains A, BALB/c, CBA, DBA, or C3H. Each point represents the average colony count of at least 20 spleens.

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cell doses were given. No spleen colonies were observed following infusion of up to and including 26×10^6 rat bone marrow cells into 1100 R irradiated (C57BL/6 × A)F_1 hybrid recipients. However, confluent splenic repopulation was observed when mice were given huge doses, *i.e.*, 37×10^6 cells or more.

In order to verify that hemopoietic repopulation was of rat origin, karyotype analysis was performed on selected "nonresistant" strains of mice (Table I). Indeed, in strains A, C3H, and BALB/c, following supralethal irradiation and infusion of 5×10^5 rat bone marrow cells or less, karyotype analysis 8 days later showed that, in both the spleen and bone marrow, virtually 100% of the metaphases were of rat origin.

Discussion. These results demonstrate that striking "resistance" to hemopoietic repopulation by rat bone marrow exists in supralethally irradiated C57BL/6 mice and in (C57BL/6 × A)F_1 hybrids, but not in A, BALB/c, CBA, DBA, or C3H mice. This phenomenon, which we are designating as "xenogeneic resistance," bears certain similarities to various aberrant murine bone marrow transplant phenomena, in both the semi-isologous and allogeneic situations. Aberrant bone marrow transplantation was originally described in the semi-isologous (parent into F_1) grafting system but has been shown recently to extend to allogeneic transplants (7). These phenomena have been independently termed "hybrid resistance" (8), "poor growth phenomenon" (9), and "CFU repression" (10)—each term connotating a different mechanism of action for the same experimental grafting results. The characteristics which are common to the xenogeneic and allogeneic (including the semi-isologous) phenomena are: (a) all are extremely radioresistant, and cannot be abrogated with supralethal (900–1100 R) irradiation; (b) all have been described for hemopoietic transplants; (c) all can be overridden by huge donor hemopoietic cell doses. The very marked similarities between the allogeneic and xenogeneic phenomena suggest that they may be manifestations of a more universal mechanism for hemopoietic (and possibly leukemic) graft failure.

TABLE I. Karyotype Analysis of "Nonresistant"[a] Strains of Mice 8 Days Following Supralethal (950–1000 R) Irradiation and Rat Marrow Transfusion.

Strain	Rat marrow cell dose ($\times 10^5$)	Percentage rat metaphases	
		Bone marrow	Spleen
A	5	98	100
C3H	1.25	100	100
BALB/c	5	98	98

[a] In "resistant" (C57BL/6 × A)F_1 mice there was no gross or microscopic evidence of hemopoietic spleen colony formation at any dose up to and including 26×10^6 cells.

Summary. A phenomenon termed "xenogeneic resistance" to hemopoietic repopulation by rat bone marrow was found to exist in supralethally irradiated (1000–1100 R) C57BL/6 mice and their F_1 hybrids, but not in mice of 5 other strains, studied by means of the spleen colony method. The similarities of this phenomenon to the phenomena known variously as "hybrid resistance," "CFU repression," or "poor growth phenomenon" suggest that these may all represent different manifestations of a single mechanism.

1. Rauchwerger, J. M., Gallagher, M. T., and Trentin, J. J., Transplantation, in press.

2. Rauchwerger, J. M., Gallagher, M. T., and Trentin, J. J., Fed. Proc., Fed. Amer. Soc. Exp. Biol. **31**, 643 (1972).

3. Rauchwerger, J. M., Gallagher, M. T., Judd, K. P., and Trentin, J. J., Transplant. Abstr. (1972).

4. Trentin, J. J., Van Hoosier, G. L., Jr., Shields, K. S., and Stenback, W. A., Lab. Anim. Care **16**, 109 (1966).

5. Curry, J. L., and Trentin, J. J., Develop. Biol. **15**, 395 (1967).

6. Utsumi, K. R., Kitamura, I., and Trentin, J. J., J. Nat. Cancer Inst. **35**, 759 (1965).

7. Cudkowicz, G., and Bennett, M., J. Exp. Med. **134**, 83 (1971).

8. Cudkowicz, G., and Stimpfling, J. H., Immunology **7**, 291 (1964).

9. Goodman, J. W., and Wheeler, H. B., Transplantation **6**, 173 (1968).

10. McCulloch, E. A., and Till, J. E., J. Cell. Comp. Physiol. **61**, 301 (1963).

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