Hybrid Resistance to Parental Marrow Grafts: Association with the K Region of H-2

Abstract. *Bone marrow of homozygous C57BL/10 donors was tested for growth in x-irradiated isogenic recipient mice, in (C57BL/10 × 129)F₁ mice, and in F₁ hybrids from congenic parents differing respectively, for an allelic substitution at one of the histocompatibility loci* H-1, H-2, H-3, *and* H-4. *All the tested* H-2 *heterozygotes resisted the transplantation of 10^6 parental marrow cells, whereas* H-2 *homozygotes were nonresistant, in spite of heterozygosity, at one or several other* H *loci. Findings in F₁ hybrids between C57BL/10 mice and mice carrying alleles resulting from crossing-over within the* H-2 *locus demonstrated that the hybrid resistance was associated with heterozygosity in the K region, but not in the D region of the* H-2 *locus.*

A number of investigators have reported that transplanted hemopoietic cells of several C57BL sublines ($H\text{-}2^b/H\text{-}2^b$) grow less well in F₁ hybrid recipients than in those of the strain of origin or in irradiated mice of allogeneic strains. In contrast, hemopoietic cells from A ($H\text{-}2^a/H\text{-}2^a$) and C3H ($H\text{-}2^k/H\text{-}2^k$) mice grow as well in F₁ as in isogenic recipients (*1*, *2*). The relative success or failure of short-term C57BL/10 marrow grafts in segregating back-cross progeny—that is, in offspring from (C3H × C57BL/10)F₁ and (A × C57BL/10)F₁ females mated to C57BL/10 males, indicated that "hybrid resistance," that is, lack of growth of viable parental cells on transplantation, requires heterozygosity at the complex histocompatibility-2 (*H-2*) locus (*2*). Furthermore, hybrid resistance to the C57BL/10 marrow could be enhanced or abolished by prolonged exposure of the resistant mice to C57BL/10 tissue antigens given as injections of viable marrow cells in the former case or viable spleen cells in the latter (*3*). Similar treatment with hemopoietic cells of the other parental strain did not elicit these responses, suggesting that the multiple injections of C57BL/10 cells induced specific immunity or specific unresponsiveness, respectively, toward C57BL/10 antigen(s) not present in the F₁ hybrid mice (*3*). Collectively, these findings pointed to the possible occurrence in *H-2* heterozygotes of genetic interaction which, presumably, prevented or modified the expression of one or more parental isoantigens. The data are consistent with the supposition that the genetic factor(s) associated with hybrid resistance lies either within or closely adjacent to the *H-2* locus of linkage group IX.

In this study, the role of several other histocompatibility loci in determining the fate of parent-to-hybrid marrow grafts was investigated with the aid of F₁ hybrid recipients from crosses between C57BL/10ScSn females and male mice of several isogenic-resistant strains and of the 129/Rl ($H\text{-}2^b/H\text{-}2^b$) strain (*4*). Each of the isogenic-resistant lines was congenic with C57BL/10 except for differences at the *H-1*, *H-2*, *H-3*, and *H-4* histocompatibility loci, respectively (*5*, *6*).

The experimental animals were exposed to 700 r of 300 kv (peak) whole-body x-rays at the age of 12 to 15 weeks and injected intravenously with 10^6 nucleated C57BL/10 marrow cells a few hours later. Five days after injection of the marrow, its growth was assayed in each of the recipients by measuring the uptake of 5-iodo-2′-deoxyuridine-I^{131} (I^{131}UdR), a specific deoxyribonucleic acid precursor, in the spleen (*7*). For this assay, each recipient was given an intraperitoneal injection of 0.5 μc of I^{131}UdR with a specific activity of ~ 100 mc/mg (*8*), and 6 to 17 hours later it was killed, its spleen removed, and the incorporated I^{131} radioactivity measured by means of crystal scintillation counting. Under the conditions used, the incorporation of I^{131}UdR into newly synthesized splenic DNA of isogenic recipient mice was a linear function of the donor marrow dose from 0.1×10^6 to 1.6×10^6 transplanted cells (*2*). Variables such as the sex of the donors and recipients, the magnitude of the x-ray exposure to the recipients, and the time between grafting and labeling with I^{131}UdR were controlled within appropriate conditions as determined previously (*2*).

In four different F₁ hybrids heterozygous at the *H-1*, *H-3*, and *H-4* loci, respectively, but homozygous for $H\text{-}2^b$, the growth of injected C57BL/10 marrow cells was indistinguishable from that in isogenic recipient mice (Table 1). The same result was obtained with (C57BL/10 × 129)F₁ mice, heterozy-

Table 1. Growth of C57BL/10(B10) marrow cells grafted into F_1 hybrids from crosses between B10 and isogenic-resistant mice differing with respect to the histocompatibility loci, *H-1*, *H-2*, *H-3*, and *H-4*.

F₁ hybrid recipients*	Heterozygosis for H loci	No. of mice	Mean uptake of I^{131}UdR (%)†	Classification
B10 × B10.BY	$H\text{-}1^c/H\text{-}1^d$	11	0.80 ± .07	Susceptible
B10 × B10.129(5M)	$H\text{-}1^c/H\text{-}1^e$	15	.71 ± .10	Susceptible
B10 × B10.LP	$H\text{-}3^a/H\text{-}3^b$	10	.67 ± .09	Susceptible
B10 × B10.129(21M)	$H\text{-}4^a/H\text{-}4^b$	12	.88 ± .08	Susceptible
B10 × 129‡	Several loci except H-2	10	.78 ± .07	Susceptible
B10 × B10.D2	$H\text{-}2^b/H\text{-}2^d$	20	.02 ± .004	Resistant
B10 × B10.A§	$H\text{-}2^b/H\text{-}2^a$	17	.03 ± .01	Resistant
B10 × B10.BR§	$H\text{-}2^b/H\text{-}2^k$	13	.04 ± .01	Resistant
B10	None	10	.76 ± .06	Susceptible

* Donor and recipient mice were females. † Mean uptake values for spleens of mice injected with marrow are given as the percentage of the total I^{131}UdR radioactivity administered ± standard error of the mean, *above* the percentage retention of radioactivity in spleens of irradiated control animals not injected with marrow. ‡ The 129/Rl strain is not congenic with B10. § For description of these lines see reference (*5*).

Table 2. Growth of C57BL/10(B10) marrow cells grafted into F_1 hybrids from crosses between B10 and mice differing for regions of *H-2*.

F₁ hybrid recipients*	Heterozygosis for H-2 components	No. of mice	Mean uptake of I^{131}UdR (%)†	Classification
B10 × Recombinant type 1	D M C H	11	0.73 ± .08	Susceptible
B10 × Recombinant type 2	C H K	10	.02 ± .008	Resistant
B10 × B10.A	D M C H K	10	.03 ± .007	Resistant
B10	None	10	.80 ± .05	Susceptible

* Donors were females; recipients were of both sexes. For description of these lines see text and reference (*5*). † Mean uptake values for spleens of mice injected with marrow are given as the percentage of the total I^{131}UdR radioactivity administered ± standard error of the mean, *above* the percentage retention of radioactivity in spleens of irradiated control animals not injected with marrow.

gous at several *H* loci, but homozygous *H-2*^{b} (Table 1). Therefore, such F_1 hybrids were considered nonresistant to the parental C57BL/10 marrow graft. On the other hand, mice that were heterozygous at the *H-2* locus, but homozygous at other *H* loci, showed deficient growth of grafted C57BL/10 marrow and were classified as resistant (Table 1). No difference among them in strength of resistance was detected, since in all instances 10^6 transplanted C57BL/10 cells failed to grow, but the possible existence of differences could be tested by grafting serial dilutions of marrow cells.

It has been shown that *H-2* is a complex gene locus including at least four regions denoted in order of linkage as D, C, V and K (*6*, *9*). Because of the demonstrated association of hybrid resistance with heterozygosity at the *H-2* locus, it was of interest to establish whether heterozygosity of the entire *H-2* locus was necessary for the manifestation of resistance to grafted C57BL/10 marrow. In the course of studying the genetic structure of *H-2* (*6*), several variants were identified and were presumably derived from cross-overs within *H-2*. The exceptional alleles were found in the offspring of *H-2*^{a}/*H-2*^{b} heterozygotes in which *H-2*^{a} was from strain A and *H-2*^{b} from C57BL/10. The *H-2* "recombinant" alleles were transferred, by repeated backcrosses, to a genetic background approximately congenic with C57BL/10. A preliminary account of these recombinants has been reported (*6*) and a more detailed description is forthcoming.

Two of the recombinant lines congenic with C57BL/10, were used in an experiment described here. The serotype of mice homozygous for the *H-2*^{a} allele is D+M+C+H+K+ (*6*) while the serotypes of the two recombinant lines are either D+M+C+H+K− (type 1) or D−M−C+H+K+ (type 2) and resemble the *H-2*^{i} and *H-2*^{h} alleles described by Gorer and Mikulska (*9*). The components of specificities M and H are thought to be closely associated with the determinants of D and V (*6*, *9*). F_1 hybrids from mice which possess the type 1 or type 2 recombinant alleles and from C57BL/10 are heterozygous for either the D or K regions of the *H-2* locus, respectively. When tested by the I^{131}UdR method, the hybrids heterozygous for the K region of *H-2* were resistant and indistinguishable from resistant *H-2*^{a}/*H-2*^{b} F_1 mice, while hybrids heterozygous for the D region of *H-2* were not resistant to C57BL/10 marrow grafts (Table 2).

Thus, C57BL/10 mice possess in the K region of *H-2* a genetic determinant whose expression is required for the growth of transplanted marrow cells. F_1 hybrid mice heterozygous for this determinant are incompatible for transplanted C57BL/10 hemopoietic cells; for example, they do not support optimal growth of minimal numbers of infused C57BL/10 marrow cells. Allogeneic strains homozygous for *H-2* alleles other than *H-2*^{b} do not display such resistance (*1*, *2*, *10*), a finding that is contrary to expectation if the factor associated with *H-2*^{b} of C57BL/10 were recessive. The long-term persistence of non-minimal C57BL marrow grafts may, however, be controlled by factors different than the *H-2* locus, not necessarily genetic in nature, especially when sufficient cells are initially transplanted to override the hybrid resistance (*11*).

It was reported that transplanted C57BL lymphomas (*5*) and sarcomas (*12*) exhibited deficient growth in F_1 hybrids. Homozygous lymphoma and carcinoma cells originating in mice of types other than *H-2*^{b}/*H-2*^{b} were also found to grow deficiently in *H-2* heterozygous F_1 hybrids (*12*, *13*). The various findings suggest that the hybrid resistance to transplantable hemopoietic cells associated with the *H-2* locus probably applies also to transplantable tumor cells. It may be inferred, therefore, that the phenomenon of hybrid resistance to parental grafts is not peculiar to hemopoietic cells or only to cells carrying *H-2*^{b}. Perhaps the relative instability of the tumor cell genome provides a variety of such noncodominant *H-2* histocompatibility factors which may be useful for elucidating the inheritance of histocompatibility in more stable cell lines.

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References and Notes

1. E. A. Boyse, *Immunology* **2**, 170 (1959); E. A. McCulloch and J. E. Till, *J. Cellular Comp. Physiol.* **61**, 301 (1963).
2. G. Cudkowicz and J. H. Stimpfling, *Genetics* **48**, 886 (1963); ———, *Immunology* **7**, 291 (1964).
3. G. Cudkowicz and J. H. Stimpfling, *Federation Proc.* **23**, 202 (1964).
4. Pedigreed breeders of the isogenic-resistant lines were made available by G. D. Snell.
5. G. D. Snell, *J. Natl. Cancer Inst.* **21**, 843 (1958); ——— and L. C. Stevens, *Immunology* **4**, 366 (1961).
6. J. H. Stimpfling and G. D. Snell, in *International Symp. Tissue Transplantation* (Universidad de Chile, Santiago, 1962) p. 37; J. H. Stimpfling, in preparation.
7. G. Cudkowicz, A. C. Upton, L. H. Smith, G. D. Gosslee, W. L. Hughes, *Ann. N.Y. Acad. Sci.* **114**, 571 (1964).
8. Nuclear Science and Engineering Co.
9. S. L. Allen, *Genetics* **40**, 627 (195➡ D. B. Amos, P. A. Gorer, Z. B. Mikulska, *Proc. Roy. Soc. London Ser. B* **144**, 369 (1955); ➡ P. A. Gorer and Z. B. Mikulska, *ibid.* **151**, 57 (1959); O. Pizzarro, G. Hoecker, P. Rubinstein, A. Ramos, *Proc. Natl. Acad. Sci. U.S.* **47**, 1900 (1961).
10. G. Cudkowicz and J. H. Stimpfling, unpublished observations.
11. R. A. Popp, *J. Natl. Cancer Inst.* **26**, 629 (1961); R. A. Popp, G. E. Cosgrove, D. M. Popp, *Ann. N.Y. Acad. Sci.* **114**, 538 (1964); R. A. Popp, *J. Natl. Cancer Inst.*, in press.
12. K. E. Hellström, *Science* **143**, 477 (1964).
13. ———, *Nature* **199**, 614 (1963).
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Intercellular Channels in the Salt-Secreting Glands of Marine Turtles

Abstract. *Long, pleomorphic microvilli project from the walls of adjacent secretory cells in the lacrymal glands of sea turtles, and a substance identified histochemically as a mucopolysaccharide fills the intercellular channels. These features are not characteristic of the principal secretory cells in the salt glands of marine birds.*

The extra-renal glands for salt secretion in marine birds and reptiles have different embryonic origins; those of marine birds are modified nasal glands (*1*), while those of reptiles are modified lacrymal glands (*2*). In both classes of vertebrates the glands secrete a sodium chloride solution that is hypertonic to blood (*3*, *4*) and on histological examination they show essentially the same pattern of organization (*3*, *5*). However, electron microscopic and cytochemical techniques disclose unique intercellular channels in the reptilian salt glands, that are not present in the salt glands of marine birds (*5–7*). These differences may reflect the separate embryonic origins of the glands, and provide two possible designs for cells in which electrolytes are concentrated.

Tissue from the salt glands of two loggerhead (*Caretta caretta*) and four green (*Chelonia mydas*) turtles was fixed in phosphate-buffered 2-percent osmium tetroxide (*8*) and dehydrated with acetone; the fixed tissue was embedded in Epon (*9*), sectioned, and