INVOLVEMENT OF THE K AND I REGIONS OF THE H-2 COMPLEX IN RESISTANCE TO HEMOPOIETIC ALLOGRAFTS

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Murine hemopoietic stem cells are able to grow and differentiate in the spleen of lethally irradiated syngeneic recipients. The growth of bone marrow grafts, however, is often suppressed in allogeneic irradiated recipients, despite the fact that such animals are immunologically deficient and do not mount allograft responses to other tissues. Moreover, hemopoietic grafts are often resisted also by irradiated F_1 hybrid recipients, whereas even nonirradiated F_1 hybrids accept parental skin grafts (1–3). This phenomenon of allogeneic and hybrid resistance to hemopoietic grafts is regulated by a complex mechanism that is yet poorly understood (4). Thus, H-2^b parental bone marrow cells fail to grow in different (H-2^b × non-H-2^b) crosses. Surprisingly, these F_1 hybrids have been shown not to resist grafts from non-H-2 parents such as C3H or BALB/c (5–8). Moreover, resistance to H-2^b parental grafts has been noted only when such F_1 hybrids were heterozygous at the H-2D region (9).

Involvement of the H-2 gene complex in a phenomenon with apparently recessive inheritance represents a difficult genetic puzzle (10). Cudkowicz (9) suggested that special recessive, tissue-specific hemopoietic histocompatibility (Hh)[1] genes, rather than classic histocompatibility genes, determine the fate of hemopoietic grafts. He further assumed that H-2^b homozygotes carry the H-2D-linked Hh-I^a allele, that H-2^k and H-2^d mice carry the Hh-I^o allele, and that the Hh-I^a/Hh-I^o heterozygotes do not express the Hh-I^a allele. This arrangement creates a peculiar situation where the H-2^b parent possesses a certain transplantation antigen that its F_1 hybrids lack (9). This hypothesis implies that hemopoietic grafts from any donors carrying the H-$2D^b$ allele will be resisted as strongly as H-2^b parental grafts, whereas grafts from H-$2D^k$- and H-$2D^d$-carrying donors will not be resisted by (H-2^b × H-2^k)F_1 and (H-2^b × H-2^d)F_1 recipients, respectively.

Testing this prediction, we found evidence that the K and I regions of H-2 are involved in hemopoietic resistance in strain combinations in which involvement of the D region had already been demonstrated by others. Moreover, we show that in some of these strain combinations, unique hybrid Ia products are

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[1] *Abbreviations used in this paper:* CFU-S, colony-forming unit/spleen; Hh, hemopoietic histocompatibility; MHC, major histocompatibility complex; PBS, phosphate-buffered saline.

 J. Exp. Med. © The Rockefeller University Press · 0022-1007/84/04/1070/13 $1.00
Volume 159 April 1984 1070–1082

responsible for hybrid resistance. The involvement of transcomplementation in hybrid resistance may suggest that the preferential association of major histocompatibility complex (MHC)-encoded molecules is responsible for the absence of parental-specific determinants in F_1 hybrids.

Materials and Methods

Mice. C57BL/6 (B6), C57BL/10 (B10), BALB/c, C3H/eb (C3H), (B6 × C3H)F_1, (B6 × BALB/c)F_1, (B10.HTG × B6)F_1, and (B10.HTG × BALB/c)F_1, and the recombinant strains listed in Table I were obtained from the Animal Breeding Center of the Weizmann Institute of Science.

Cell Preparation. Bone marrow cells were obtained from 2–3-mo-old mice by flushing the tibia and femur into phosphate-buffered saline (PBS). Cells were centrifuged, the pellet was resuspended in NH_4Cl buffer to lyse erythrocytes, washed twice, resuspended in PBS, and counted.

Hemopoietic Stem Cell Assay. Colony-forming units/spleen (CFU-S) were determined using the method of Till and McCulloch (11). Briefly, the recipient mice were total-body irradiated (850 rad) from a cobalt-60 source and injected intravenously with varying numbers (10^4–10^7) of bone marrow cells. 8 d later, the animals were killed and their spleens removed and fixed in Bouin's solution. After 2 h of fixation, macroscopically visible spleen colonies were counted.

The number of bone marrow cells needed to overcome hemopoietic resistance was determined in most donor-recipient combinations. Groups of 8–12 mice were injected with a given marrow cell dose, and arithmetic means and standard deviations were calculated from individual spleen counts. Negative controls were irradiated mice that received no marrow cells. In all cases, the background spleen colony formation was no more than 0.2. For positive controls, bone marrow cells were injected into irradiated syngeneic recipients.

Results

In preliminary studies we measured the strength of hybrid resistance in two parental-F_1 combinations by determining the number of bone marrow cells needed to overcome it, rather than registering the presence or absence of the phenomenon after injecting a single high dose of bone marrow cells (0.5×10^6 to 10^6), as had been done by others (3). (B6 × C3H)F_1 and (B6 × BALB/c)F_1 irradiated recipients were injected with various numbers of syngeneic hybrid and parental marrow cells (Fig. 1). As a control, syngeneic transplantation was

TABLE I
Genetic Composition of Strains Used

Strain	Haplotype	K	Aα	Aβ	Eβ	J	Eα	S	D
B10.A (2R)	h2	k	k	k	k	k	k	d	b
B10.A (4R)	h4	k	k	k	k	b	b	b	b
B10.HTG	g	d	d	d	d	d	d	d	b
D2.GD	g2	d	d	d	d/b	b	b	b	b
B10.A (5R)	i5	b	b	b	b	k	k	d	d
A.AL	a1	k	k	k	k	k	k	k	d
A.TL	t1	s	k	k	k	k	k	k	d
A.TFR-5	ap5	f	f	f	f	f	k	k	d
C3H.OH	o2	d	d	d	d	d	d	d	k

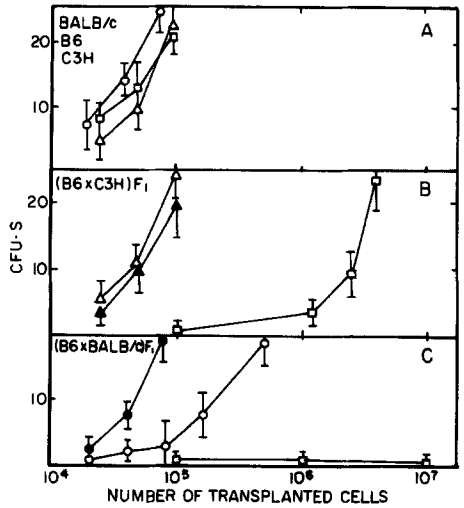

FIGURE 1. Variability of hybrid resistance. CFU-S formation by irradiated BALB/c, B6, and C3H (*A*), (B6 × C3H)F_1 (*B*), and (B6 × BALB/c)F_1 (*C*) recipients injected with graded doses of bone marrow cells from BALB/c (○) B6 (□), C3H (△), (B6 × C3H)F_1 (▲), and (B6 × BALB/c)F_1 (●) donors.

performed (Fig. 1 *A*), yielding similar results with all the strains used throughout this study (see Figs. 2–5).

Experiments with F_1 recipients gave somewhat unexpected results. Namely, a remarkable difference was observed in the strength of resistance of the above two F_1 recipients to the parental grafts. Whereas 4×10^6 B6 bone marrow cells were needed to overcome hybrid resistance to (B6 × C3H)F_1 hosts (Fig. 1 *B*), even 10^7 of the same marrow cells failed to grow in (B6 × BALB/c)F_1 hosts (Fig. 1 *C*). Moreover, significant resistance of (B6 × BALB/c)F_1 hybrids to 2×10^4 to 10^5 BALB/c parental marrow cells was observed (Fig. 1 *C*), whereas the same number of C3H parental cells grew unresisted in (B6 × C3H)F_1 hybrids (Fig. 1 *B*).

As seen in Fig. 1 *C*, the growth of 0.5×10^6 BALB/c parental marrow cells was not resisted by (B6 × BALB/c)F_1 recipients. This agrees with the findings of others (5–8) who used the same or higher cell doses. It appears, however, that this amount of cells grew unresisted because they were able to overcome hybrid resistance to BALB/c parental grafts, which would have been revealed by the sensitive assay we used. It permits the detection of spleen colony formation in a much lower dose range (10^4–10^5).

We repeated these experiments four times with virtually similar results. Hybrid resistance was observable against both parental strains, although to a different degree, suggesting that the strength of resistance to H-2^b parental grafts by F_1 hybrids depends on the genetic constitution of the input strains. Hence, these data raise the question whether H-2D-linked loci are the only factor determining this variable phenomenology.

Are Loci of the H-2D Region Solely Responsible for Hybrid Resistance? To check whether H-2D-linked loci are the main and only factor determining the fate of parental hemopoietic grafts, we measured the strength of resistance of the same

F_1 recipients used above toward marrow grafts from a set of H-2 recombinants and F_1 hybrids carrying the H-2D^b, H-2D^d, and H-2D^k alleles, respectively. Thus, we were able to assess the role of the left part of the H-2 complex in hybrid resistance.

Irradiated (B6 × BALB/c)F_1 and (B6 × C3H)F_1 recipients were injected with graded doses of bone marrow cells from a series of H-2 recombinant strains all of which carry the H-2D^b allele (Fig. 2). It can be seen in Fig. 2*B* that up to 10^7 B10.A(4R) or B10.A(2R) marrow cells were resisted by the (B6 × BALB/c)F_1 recipients, whereas in (B6 × C3H)F_1 recipients (Fig. 2*C*), the smaller dose of 10^6 cells from these strains gave values similar to the syngeneic controls (Fig. 2*A*). The opposite picture was observed with B10.HTG and D2.GD grafts. They were strongly resisted only by (B6 × C3H)F_1 recipients (Fig. 2*C*) and much better tolerated by (B6 × BALB/c)F_1 recipients (Fig. 2*B*).

The data show that all four H-2D^b grafts were resisted, but to different degrees, supporting the conclusion of others (5, 6) that loci within or close to the H-2D region are responsible for hybrid resistance to H-2^b parental grafts. It is to be noted that differences in the strength of resistance cannot be attributed to the H-2D^b allele because all four donors shared it.

The fact that B10.A(2R) and B10.HTG grafts, which are congenic and differ only at H-2K and H-2I, behave differently, whereas B10.HTG and D2.GD, which share H-2K and H-2A but differ in their background, behave similarly, rules out the possibility that genes outside the H-2 have a significant effect in these experiments. Hence, the data suggest the involvement of the left part of

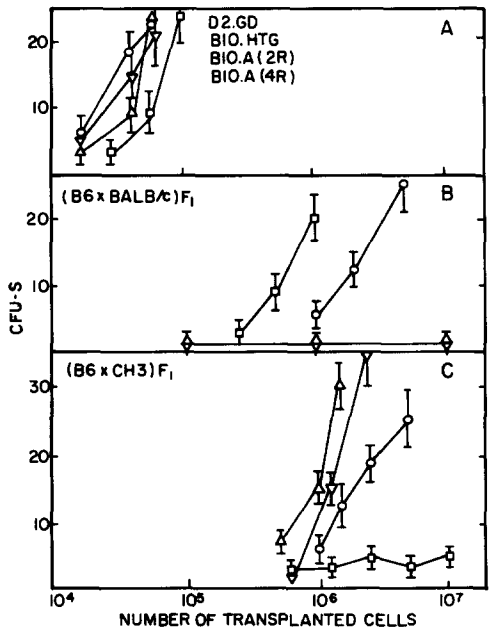

FIGURE 2. The H-2D^b allele and hybrid resistance. CFU-S formation by irradiated syngeneic (*A*), (B6 × BALB/c)F_1 (*B*), and (B6 × C3H)F_1 (*C*) recipients injected with graded doses of bone marrow cells from the H-2D^b allele-carrying donors: D2.GD (□), B10.HTG (○), B10.A(2R) (△), and B10.A(4R) (▽).

the H-2 complex in hybrid resistance. Moreover, the recipients showed the strongest resistance, similar in strength to that against B6 parental grafts, toward the recombinant donor, which was fully mismatched relative to the input strains, e.g., B10.A(2R) and B10.A(4R) with BALB/c in the (B6 × BALB/c)F_1 recipients and B10.HTG and D2.GD with C3H in the (B6 × C3H)F_1 recipients. Conversely, when the donor shared with the input strain the H-2K and H-2I regions, e.g., in B10.A(2R) and B10.(4R) vs. C3H, or B10.HTG and D2.GD vs. BALB/c, the resistance was considerably weaker.

The data suggest that the strength of resistance of F_1 hybrids to hemopoietic grafts from donors that share the H-2D allele with the H-2^b parent is considerably influenced by the H-2K and/or H-2I regions. They also imply that strong resistance to B6 parental grafts might be attributed to disparities at both H-2D and H-2K or H-2I regions between B6 parents and BALB/c or C3H input strains.

To further elucidate the role of the H-2D region in hemopoietic resistance, we explored the above described observation of weak resistance to BALB/c parental grafts (Fig. 1 *C*). (B6 × BALB/c)F_1 recipients were injected with graded doses of bone marrow cells from four H-2 recombinant strains, all of which carry the H-$2D^d$ allele, and with BALB/c parental marrow cells (Fig. 3*B*). It can be seen that A.TFR-5 and BALB/c grafts were resisted, whereas A.AL, A.TL, and B10.A(5R) cells grew unresisted, as in syngeneic recipients (Fig. 3*A*). These data show that, unlike the H-$2D^b$ allele, expression of the H-$2D^d$ allele by recombinant donors does not necessarily lead to rejection of hemopoietic grafts by (B6 × BALB/c)F_1 recipients. The different fate of H-$2D^d$ grafts in these experiments also cannot be attributed to differences in the genetic background, since grafts from congenic A.AL and A.TFR-5 donors were accepted and rejected, respec-

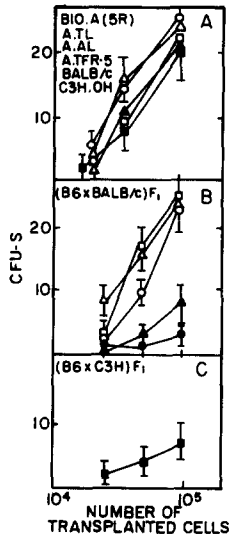

FIGURE 3. The H-$2D^d$ and H-$2D^k$ alleles and hybrid resistance. CFU-S formation by irradiated syngeneic (*A*), (B6 × BALB/c)F_1 (*B*), and (B6 × C3H)F_1 (*C*) recipients injected with graded doses of bone marrow cells from B10.A(5R) (○), A.TL (□), A.AL (△), A.TFR-5 (▲), BALB/c (●), and C3H.OH (■) donors.

tively, whereas grafts from donors with different background, namely from B10.A and A.AL or A.TL, were accepted. The only factor in these experiments that correlated with the acceptance of H-D^d grafts by (B6 × BALB/c)F_1 recipients is combinatorial specificity Ia.22. The latter was shown to be formed by the I-E^k molecule, born by both A.AL and A.TL, and also by ciscomplementation (E_α^k:E_β^b) in B10.A(5R) or by transcomplementation (E_α^d:E_β^b) in (H-2^b × H-2^d)F_1 hybrids. Neither BALB/c nor A.TER-5 forms this determinant (12–15). Hence, the data shown in Fig. 3*B* may tentatively suggest the involvement of the class II MHC products in hemopoietic resistance.

In the last experiment shown (Fig. 3*B*), congenic C3H.OH bone marrow cells were injected into (B6 × C3H)F_1 irradiated recipients. Unlike the C3H parental cells, these cells were resisted by the F_1 host. Since C3H and C3H.OH share genetic background and H-2D, the resistance observed can be attributed to the H-2K and H-2I regions of the complex.

Allogeneic Resistance in the H-2D Compatible Donor Recipient Combinations. Previous experiments in which F_1 recipients were grafted with marrow cells from H-2 recombinants represent a rather complicated variant of allotransplantation. Studies of a simple homozygous strain combination, where neither recessive inheritance nor transcomplementation took place, suggest that allogeneic resistance to hemopoietic grafts is caused by H-2D disparities only. In these experiments, in which a relatively high dose of bone marrow cells was used (0.5×10^6 to 10^6 cells), no resistance was observed in H-2D compatible, H-2K, or H-2I incompatible donor-recipient combinations (16).

In the present work, we reinvestigated allogeneic resistance and used lower bone marrow cell doses (5×10^4 to 10^5). Fig. 4 demonstrates that in this more sensitive experimental arrangement, significant resistance was observed in three congenic, recombinant, H-$2D^b$-identical strain combinations, suggesting the involvement of the H-2K and H-2I regions not only in hybrid but also in allogeneic resistance.

Unique Hybrid Class II Antigens and Hemopoietic Resistance. The results in Fig. 3 suggested that unique Ia products in F_1 hybrids and their absence in the parental donor may facilitate hybrid resistance. To get a better insight into the problem, we prepared F_1 crosses between B10.HTG and BALB/c and used them as irradiated recipients of graded doses of BALB/c bone marrow cells. As a control, the same BALB/c cells were injected into (B6 × BALB/c)F_1 recipients.

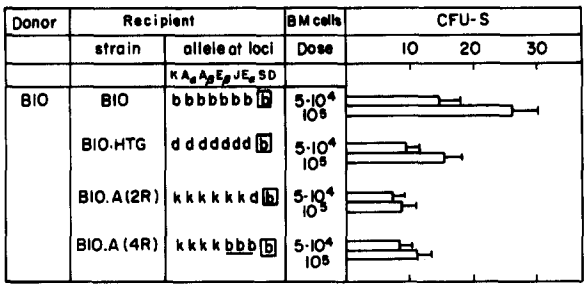

FIGURE 4. The H-$2D^b$ allele and allogeneic resistance. Growth of B10 bone marrow (BM) cells in irradiated syngeneic and congenic recombinant, H-2D compatible recipients.

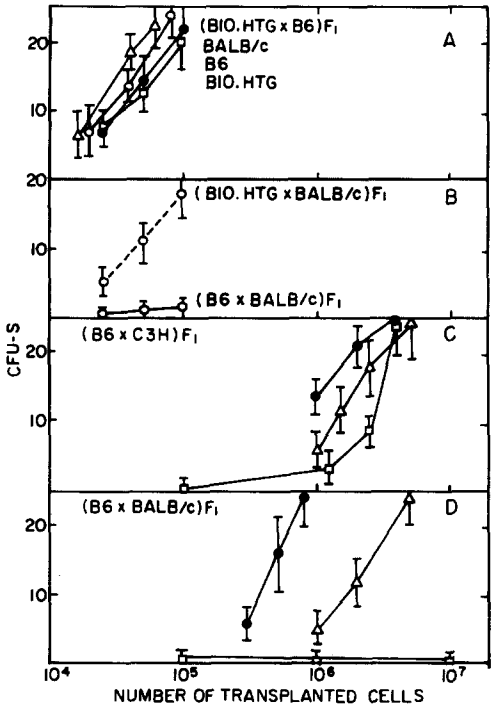

FIGURE 5. The unique hybrid Ia molecules and hybrid resistance. CFU-S formation by (*A*) irradiated syngeneic recipients; (*B*) irradiated (B10.HTG × BALB/c)F_1 (○--○) and (B6 × BALB/c)F_1 (○—○) recipients injected with BALB/c parental bone marrow cells; (*C*) irradiated (B6 × C3H)F_1 and (*D*) (B6 × BALB/c)F_1 recipients injected with graded doses of bone marrow cells from (B10.HTG × B6)F_1 (●), B10.HTG (△), and B6 (□) donor cells.

Fig. 5*B* shows that unlike (B6 × BALB/c)F_1, (B10.HTG × BALB/c)F_1 recipients tolerated the BALB/c parental grafts. We attributed this absence of resistance to the fact that in (B10.HTG × BALB/c)F_1, unlike in (B6 × BALB/c)F_1, no unique F_1 hybrid determinants are formed, because the parents carry identical I-A and I-E alleles.

Next, the possible involvement of unique Ia products was approached using a strain combination where the H-2D^b allele could contribute to the strength of hemopoietic resistance. H-2D^b homozygous (B10.HTG × B6)F_1 hybrid bone marrow cells were injected into irradiated (B6 × C3H)F_1 or (B6 × BALB/c)F_1 recipients. As the control, parental B10.HTG and B6 marrow cells were injected into similar recipients. Syngeneic control groups also were included in these experiments (Fig. 5*A*).

Fig. 5*C* shows that (B10.HTG × B6)F_1 donor cells grew in the (B6 × C3H)F_1 recipients somewhat better than B10.HTG or B6 parental donor cells. In contrast, in the (B6 × BALB/c)F_1 recipients, the growth of (B10.HTG × B6)F_1, B10.HTG, and B6 grafts differed significantly. As shown in Fig. 5*D* (B10.HTG × B6)F_1 grafts were accepted by the (B6 × BALB/c)F_1 recipients at an almost 10-fold lesser cell dose than B10.HTG grafts. An even much higher dose difference was observed between (B10.HTG × B6)F_1 and B6 grafts.

Since the combinations are syngeneic for non-H-2 genes, this different behav-

ior of parental B10.HTG and B6 vs. (B10.HTG × B6)F_1 hybrid donors can be explained only by complementation in the (B10.HTG × B6)F_1 donor cells, which had to occur between H-2^d and H-2^b alleles and may have been due to transcomplementation of the respective parental Ia products (A_α:A_β and E_α:E_β) (17). Hence, a different behavior of the (B10.HTG × B6)F_1 donor grafts in the (B6 × C3H)F_1 vs. (B6 × BALB/c)F_1 recipient may be attributed to the fact that (B10.HTG × B6)F_1 donors and (B6 × C3H)F_1 recipients form different hybrid Ia products (except for the Ia.22 specificity), whereas (B10.HTG × B6)F_1 donors and (B6 × BALB/c)F_1 recipients form the identical unique hybrid Ia products.

Taken together, the experiment shown in Figs. 3 and 5 suggest the involvement of unique hybrid class II antigens in hemopoietic resistance.

Discussion

Resistance to nonsyngeneic hemopoietic grafts violates fundamental laws of transplantation mainly because of radiation resistance and apparent recessive inheritance. This led Cudkowicz and his colleagues to the conclusion that not the classic but special H-2D-linked recessive hemopoietic histocompatibility genes control hemopoietic resistance.

Here we investigated the quantitative aspects of hemopoietic resistance to grafts carrying the H-$2D^b$, H-$2D^d$, and H-$2D^k$ alleles, respectively. The data we obtained cannot be explained by the assumption that an H-2D-associated recessive allele is the main and only factor of hemopoietic resistance in these experiments. Our data suggest that genes outside the H-2D, possibly in the left part of the H-2 complex, are also responsible. This assumption is based on the following findings: (*a*) Grafts carrying the H-$2D^b$ allele were resisted by congeneic, H-2D-identical, H-2K- and H-2I-incompatible recipients (Fig. 4). The strength of resistance of (B6 × BALB/c)F_1 and (B6 × C3H)F_1 recipients was weak, when H-$2D^b$ donors shared H-2K and H-2I alleles with the input strain of the F_1 recipient (Fig. 2), or when the donors and the recipients formed identical unique hybrid class II antigens (Fig. 5*D*). (*b*) The fate of grafts from H-D^d donors seems to depend on the incompatibility of a combinatorial determinant Ia.22. If both donor and recipient expressed such a hybrid determinant (either in the *cis* or in the *trans*position), or if neither could form such determinants, grafts were not resisted (Figs. 3*B* and 5*B*). (*c*) The H-$2D^k$ allele is not the only factor that confers to C3H parental bone marrow cells the ability to grow unresisted in (B6 × C3H)F_1 recipients, since grafts from congeneic C3H.OH donors, carrying the same H-$2D^k$ allele and differing at the left part of the H-2 complex, were resisted (Fig. 3*C*).

Taken together, these data suggest the involvement of the H-2K and H-2I regions in hemopoietic resistance and raise the possibility that the A_α, A_β, E_α, and E_β genes rather than some other H-2I-linked loci are involved. This conclusion agrees with the recent study of the molecular map of the H-2 complex, which revealed that the I region has no room for any yet unknown genes (18).

Our observations agree with some findings of others. Involvement of the H-2K and H-2I regions in hybrid resistance to tumor cells, a phenomenon related to hemopoietic resistance, was recently reported. It has been shown that the strength of resistance is affected by mutations of the H-$2K^b$ and H-$2A^b$ genes

(19). Bennett (20) found that in some situations, F_1 hybrid recipients accepted marrow allografts from heterozygous but not from homozygous donors. Thus, (B10 × C3H)F_1 recipients rejected both B10 parental and DBA/2 or WB allogeneic marrow grafts. The F_1 hybrids, however, accepted (B10 × DBA/2)F_1 but not (WB × DBA/2)F_1 marrow grafts. This phenomenon resembles our present observation with (B10.HTG × B6)F_1 donor cells (Fig. 5*D*) and may be generally attributed to the fact that different heterozygotes can share hybrid Ia molecules, which neither of the homozygous parents express. On the other hand, at least some of the H-2D-linked loci that have been shown to control hemopoietic resistance appeared to be classic MHC genes, since mutations of the H-2D^d and H-2L^d genes are able to alter both allogeneic (21) and hybrid (8, 22) resistance. Accordingly, F_1 hybrids were shown to generate in vitro antiparental cytotoxic T lymphocytes directed toward parental class I MHC antigens (7, 23).

Taken together, these data suggest that MHC genes, rather than special Hh genes, determine the fate of hemopoietic grafts. This conclusion, however, creates a paradox: According to the laws of transplantation, parents do not express H-2 antigens, which F_1 hybrids lack, but experimental data suggest that they do.

The phenomenon of hemopoietic resistance raises two yet unanswered questions: What is the nature of the determinants that homozygous parents are supposed to possess but their F_1 hybrids lack, and what is the mechanism that enables lethally irradiated animals to resist nonsyngeneic hemopoietic grafts?

Here we propose a hypothesis to answer these questions. We postulate that there exist parental determinants that are not formed in some F_1 hybrids due to preferential association of either Ia α chains with allogeneic β chains or class I antigens with allogeneic or hybrid class II restriction elements.

Preferential association of E_α chains with either syngeneic (24) or allogeneic (25) E_β chains in some F_1 hybrids has been recently described. The authors suggested that preferential association of Ia chains might be a general trait and might include formation of A_α:A_β complexes (25). Indeed, a strain A anti-B6 alloreactive T cell clone has been described, which could have been stimulated by B6 but not (B6 × A)F_1 cells. This implies the presence of a unique H-2^b parental MLR-stimulating determinant that is absent on heterozygous (B6 × A)F_1 cells (26). It is possible that this determinant is the A_α^b:A_β^b complex, which is not formed in (B6 × A)F_1 hybrids because of the preferential association of A_α^b chain with allogeneic A_β^k chain. Moreover, the absence of this particular determinant in (B6 × A)F_1 hybrids might possibly account for their resistance to B6 parental bone marrow grafts.

There is no evidence suggesting preferential association of class I with class II products. The existence of such complexes was postulated by Matzinger and Bevan (27). Later it was shown that Ia-positive splenic adherent cells are predominant stimulators of a mixed lymphocyte response to the non–H-2-linked Mls products or to products of the K and D regions of H-2 (28). Furthermore, monoclonal antiresponder Ia antibodies inhibited the proliferative response to class I (H-2K/D) MHC antigens (29) and both the proliferative and the cytotoxic response of B6 responders to H-2K^{ba} mutant class I antigen (30).

The question whether tolerance to these antigens is Ia restricted is still open

(31). In any case, the data indicate that class I MHC antigens can be recognized by proliferative and T helper cells in association with syngeneic Ia molecules. Hence, these antigens seem to behave like conventional soluble antigens. The latter, however, were shown to be able to associate with both *cis*complementing and *trans*complementing Ia proteins (32), and sometimes, being given a choice of several restriction elements, preferentially associated with one of them (33). Therefore, it is conceivable that certain F_1 heterozygotes do not form some of the parental-specific class I and II complexes and hence regard them as non-self.

The proposed model makes it possible to understand why F_1 hybrids are able to react in one or another way against parental tissues. However, the exact cellular mechanism of hybrid resistance is still unknown. Natural killer cells were suggested to be responsible for hemopoietic graft rejection (34). Recently (35), it has been shown that very few host T lymphocytes, which survived irradiation, were sufficient to suppress growth of hemopoietic allografts. An alternative cellular mechanism has been proposed by Lengerova and colleagues (21, 36). They presented evidence that hemopoietic allografts fail to grow due to the inability of nonsyngeneic stroma to provide an appropriate differentiative signal to hemopoietic stem cells. We assume that whichever cells are responsible for hemopoietic resistance, they either recognize class I and II complexes expressed on hemopoietic stem cells as foreign or do not recognize them as self. It is conceivable that similar mechanisms also regulate bone marrow engraftment in humans.

Summary

Irradiated (H-2^b × H-2^k)F_1 and (H-2^b × H-2^d)F_1 recipients strongly resist the growth of H-2^b parental bone marrow cells and do not resist marrow grafts from non-H-2^b parents such as C3H and BALB/c. This phenomenon of hybrid resistance has been shown to be under genetic control of the H-2D-linked loci and was interpreted by Cudkowicz (9) as due to the existence of H-2D-linked recessive hemopoietic histocompatibility genes.

To check whether the H-2D-linked loci are solely responsible for the fate of bone marrow allografts, we measured the strength of resistance of irradiated (B6 × C3H)F_1 and (B6 × BALB/c)F_1 recipients toward bone marrow grafts from a set of H-2 recombinant and F_1 hybrid donors carrying either the H-2^b, H-2^d, and H-2^k alleles.

We found that growth of all H-2^b grafts was resisted, although to different degrees. Resistance was minimal when donors shared with the input strain of a corresponding F_1 hybrid the H-2K and H-2I regions, or when both F_1 donors and F_1 recipients formed identical unique hybrid Ia molecules. In addition, H-2^b grafts were resisted by congenic, H-2D-identical, H-2K- and H-2I-incompatible recipients.

The fate of grafts from H-$2D^d$ donors seemed to depend on the incompatibility of the combinatorial determinant Ia.22. If both donor and recipient expressed such a determinant (either in the *cis* or in the *trans*position), or if neither could form such a determinant, grafts were not resisted.

The H-$2D^k$ allele is not the main or only factor that confers on the C3H parental bone marrow cells the ability to grow unresisted in (B6 × C3H)F_1

recipients. Grafts from congenic C3H.OH donors, carrying the same H-2D^k alleles and differing in the left part of the H-2 complex, were resisted by the F_1 recipients. We conclude that both class I (K and D) and class II (I-A and I-E) major histocompatibility complex genes, rather than hypothetical hemopoietic histocompatibility genes control hemopoietic resistance.

To reconcile codominant inheritance of classic H-2 antigens with the apparent recessive inheritance of hybrid resistance, we assume that there exist parental determinants that are not formed in some F_1 hybrids due to preferential association of either Ia α chains with allogeneic β chains or of class I antigens with allogeneic or hybrid class II restriction elements.

We thank Dr. I. R. Cohen and Dr. D. Zipori for their careful review of the manuscript. We are especially indebted to Dr. P. Lonai for his constant help, invaluable suggestions, and constructive criticism both in the course of these studies and during preparation of the manuscript.

Received for publication 8 August 1983 and in revised form 3 January 1984.

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