GENETIC CONTROL OF BONE MARROW GRAFT REJECTION

I. Determinant-Specific Difference of Reactivity in Two Pairs of Inbred Mouse Strains*

By GUSTAVO CUDKOWICZ, M.D.

(From the Department of Pathology, School of Medicine, State University of New York at Buffalo, Buffalo, New York 14214)

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The specificity and magnitude of humoral antibody responses (1–8) and the susceptibility of mice to leukemogenic viruses (9–15) are controlled by specific regulator genes. The mechanisms of action of such genes are not known but vary, presumably, from one response to another. The processes under genetic influence are complex and require participation of several cell types which are recruited from different differentiation pathways (6, 11–15, 32). The genetic nomenclature reflects the present status of knowledge, and thus makes use of the new operational gene symbols *Ir* (immune response), *Fv* (*Friend virus*), and *Rgv* (resistance to Gross virus), as well as of established symbols of pleiotropic genes, e.g., *H* (histocompatibility), *W* (dominant spotting), and *Sl* (steel). The most intriguing observation was the close linkage in mice and guinea pigs of *Ir* and *virus resistance* genes with *H* genes. It was suggested that this linkage may in fact be identity and that products of the *H* genes play a role in immunological "recognition" of antigens, viruses, and cell-surface antigens (1, 4, 9, 16–18). If the hypothesis is correct, allograft reactions should also be subject to genetic regulation. Investigation of this topic is in the beginning stage; strain differences in rejection of syngeneic or parental-strain male skin grafts by female mice were known since 1959 (19–23), and are now ascribed to the effect of a regulator gene linked with *H-2* or to a regulatory function of *H-2* itself (24–26).

The studies to be described are an outgrowth of the observation that given *H-2*-incompatible bone marrow grafts do not take in irradiated hosts of a number of mouse strains but do take in others, and that susceptible and resistant mice often belong to inbred strains with the same *H-2* allele (27, 28). The earlier studies established that susceptible mice fail to reject allografts for being nonresponders to given *H-2* alloantigens, whereas resistant host mice of the same *H-2* type reject the grafts for being good responders. Segregation of

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resistance and susceptibility to marrow allografts is now described among the progeny of an intercross of (C57BL/10 × 129)F_1 mice, and of a backcross of (B10.BR × C3H)F_1 mice to C3H parents. The results confirm that differences between the two *H-2*^{b} strains C57BL/10 and 129 in reactivity to DBA/2 (*H-2*^{d}) grafts, and those of the two *H-2*^{k} strains B10.BR and C3H in reactivity to C57BL/10 (*H-2*^{b}) grafts are genetically determined. Furthermore, the segregation ratios suggest that a minimum of two independent gene loci control rejection of marrow allografts in the progeny of both crosses, and that resistance is conferred by the dominant alleles.

Materials and Methods

Mice.—All inbred, F_1, F_2, and backcross mice were raised in our animal colony. Pedigreed breeders were supplied by G. D. Snell, the Jackson Laboratories, Bar Harbor, Maine (C57BL/10ScSn, abbreviated B10), J. H. Stimpfling, Columbus Hospital, Great Falls, Mont. (B10.A, B10.BR), L. B. Russell, Oak Ridge National Laboratory, Oak Ridge, Tenn. (C3H/He.*Sl*), and T. S. Hauschka, Roswell Park Memorial Institute, Buffalo, N. Y. (C3H/He, DBA/2Ha, and 129/Rr). C3H/He.*Sl* mice of genotype *Sl*/+ were congenic with C3H/He mice; the former were used for outcrosses with B10.BR and for backcrosses because the coat color of *Sl*/+ segregants provided a genetic marker of linkage group IV (29).

B10.*tf,T* mice of genotype *tf/tf,T*/+ (phenotype, tufted hair and short tail) were obtained by outcrossing inbred mice of the B10 strain to mice of a marker stock made available by L. C. Dunn, Nevis Biological Station, Columbia University, Irvington on Hudson, N. Y. The tufted (*tf*) and brachyury (*T*) alleles of the marker stock were transferred onto the B10 genetic background by 10 consecutive backcrosses of *tf*/+, *T*/+ mice to B10. Heterozygotes of the last backcross generation were intercrossed and *tf/tf*, *T*/+ siblings were inbred thereafter. At the time of these experiments B10.*tf*, *T* mice were inbred for three generations and their erythrocytes, tested by direct hemagglutination, were positive for the *H-2* alloantigens 2 and 5, and negative for 1, 3, 4, 8, 11, 23 and 31, like B10 erythrocytes.[1] However, the B10 and B10.*tf,T* lines were congenic for *tf*, *T* and some other *H* locus because exchanged skin grafts were not accepted permanently. In these experiments the mice were used for outcrosses with 129-strain mice and for F_2 intercrosses because the tufted hair and short tails of *tf/tf*, *T*/+ segregants provided genetic markers of linkage group IX (29).

F_1 hybrid, F_2 intercross, and backcross mice were designated by listing first the female and then the male parental strain, e.g., (B10.BR ♀ × C3H ♂)F_1 ♀ × C3H ♂.

Irradiation.—Mice to be grafted with marrow cells were exposed to 800–850 R of total body X-irradiation as described elsewhere (30).

Cell Suspensions, Transplantation, and Assay for Proliferation of Donor Cells.—Nucleated bone marrow cells, suspended in Eagle's medium, were counted and injected into a lateral tail vein of irradiated mice (28, 30). 5 days later, the DNA precursor 5-iodo-2′-deoxyuridine (IUdR), labeled with radioactive ^{125}I, was used to assess DNA synthesis by donor-derived cells in recipient spleens, as previously described (30). The values of IUdR uptake were expressed as per cent of injected radioactivity retained in spleens of individual mice or in spleens of groups of mice (geometric means ± standard errors). Negative controls were irradiated mice not injected with marrow cells; the uptake values of IUdR in such spleens were not

[1] Direct hemagglutination tests in polyvinylpyrrolidone were done by Dr. Eva Lotzová of this Department using "monospecific" antisera C-1b, C-2, C-3b, C-4, C-5, C-8, C-11b, C-23, and C-31 obtained from G. D. Snell through the Transplantation Immunology Branch, National Institute of Allergy and Infectious Dieasses, Bethesda, Md.

greater than 0.03%. Positive controls were irradiated mice grafted with 3–5 × 10^5 syngeneic cells; the splenic uptake values of IUdR were 0.3–0.9%.

RESULTS

Marrow Graft Failures and Takes in Host Mice of Identical H-2 Type

3–5 × 10^5 marrow cells were transplanted into syngeneic and allogeneic mice, 2–4 hr after 800–850 R of X-rays. For DBA/2 (*H-2*^{d}) donors, the allogeneic recipients were chosen from *H-2*^{b} strains (129 and B10 mice), and for B10 (*H-2*^{b}) donors the recipients were chosen from *H-2*^{k} strains (C3H and B10.BR mice). The extent of splenic repopulation was assessed 5 days after irradiation and grafting (Table I).

In both donor-host combinations, recipients of one strain accepted the *H-2*–incompatible grafts, and those of the second strain did not, as indicated by the splenic uptake values of IUdR. The differences in susceptibility to marrow allografts were substantial even though the *H-2* relationship between donor and hosts was identical for each pair of recipients. (B10 × 129)F_1 and (B10.BR × C3H)F_1 hybrids from crosses between susceptible and resistant mice of identical *H-2* type were resistant to DBA/2 and B10 grafts, respectively. This confirmed earlier experiments (28). The presence of mutant alleles at two loci in linkage groups IX and at one locus in linkage group IV did not influence the outcome of marrow allografts in the inbred and F_1 mice used in these experiments (Table I). Likewise, the sexes of donors, recipients, and of the parents entering F_1 crosses had no detectable effect. Resistance and susceptibility were specific for donor cells of given *H-2* type. B10 mice were resistant to DBA/2, but partially susceptible to C3H grafts; and 129-strain mice were susceptible to DBA/2, but resistant to B10.A (*H-2*^{a}) grafts.

Alloantigens of donor cells specified by histocompatibility loci other than *H-2* do not cause marrow graft failure in irradiated recipients (28).

Segregation of Resistance and Susceptibility to DBA/2 Grafts in F_2 Mice

(B10 × 129)F_1 and reciprocal hybrids, resistant to DBA/2 marrow grafts, were intercrossed and the progeny classified as to resistance or susceptibility. Short-tail hybrids of genotype *tf*/+, *T*/+ were mated to normal-tail hybrids of genotype *tf*/+, +/+ to avoid the lethal effect of homozygosity for the *T* allele in F_2 mice. A total of 252 F_2 animals (117 females and 135 males) were exposed to 850 R of X-rays a few hours before transplantation of 5 × 10^5 DBA/2 marrow cells from donors of the same sex. The F_2 animals were grafted in four separate experiments along with mice of the parental strains (33 B10 and 24 129-strain mice) and 40 F_1 hybrids. The parental-strain mice provided reference populations of resistant and susceptible animals with which to compare segregating F_2 mice. Results were plotted, as cumulative frequencies of grafted mice with increasing values of splenic uptake of IUdR (Fig. 1), separately for the two sexes.

In irradiated B10 and (B10 × 129)F_1 mice grafted with DBA/2 cells, the splenic uptake of IUdR ranged from 0.01 to 0.1%, and in irradiated 129-strain mice from 0.2 to 0.9%. Female and male reference populations did not overlap for IUdR uptake, but they were not separated by a wide gap. The range of

values was wider in F_2 mice than in the reference populations, from 0.01 to 0.9%. Segregants were classifiable as resistant or susceptible, and as intermediate, depending on the value of splenic uptake of IUdR. The intermediate animals were those with 0.11–0.3% uptake, a class which probably was different from the parental-strain and F_1 hybrid mice. The observed frequencies of F_2

TABLE I

Proliferation of Bone Marrow Cells in Irradiated Recipient Mice Estimated by Splenic Uptake of the DNA Precursor IUdR

Donor strain	No. of cells grafted	Recipient		No. and sex		Per cent splenic uptake of ^{125}IUdR‡ (geometric mean ± SE)
		Strain	Genotype*	♀♀	♂♂	
DBA/2	3×10^5	DBA/2		5	5	0.81 ± 0.08
		129	A^w/A^w, c^{ch}/c^{ch}, p/p	5	6	0.53 ± 0.08
		B10	tf/tf, $T/+$	4	3	0.01
		B10 × 129	$tf/+$	3	3	0.01
		129 × B10	$tf/+$	3	3	0.03
		B10 × 129	$tf/+$, $T/+$	3	5	0.03
B10	5×10^5	B10		6	10	0.46 ± 0.06
		C3H		4	4	0.50 ± 0.05
		C3H	$Sl/+$		5	0.52 ± 0.04
		B10.BR		5	5	0.02
		B10.BR × C3H		6	5	0.02
		B10.BR × C3H	$Sl/+$	5	3	0.02
C3H	5×10^5	C3H		4	5	0.44 ± 0.04
		B10			9	0.32 ± 0.03
B10.A	5×10^5	B10.A		5	9	0.35 ± 0.02
		129		5		0.05

* Marker genes to be introduced in F_2 intercrosses and backcrosses. +, wild-type allele; A^w, white-bellied agouti; c^{ch}, chinchilla; p, pink eye; tf, tufted; T, brachyury; Sl, steel.

‡ 5 days after irradiation (800 R to inbred mice and 850 R to F_1 mice) and transplantation. Radiation control values of IUdR uptake were subtracted from experimental values of susceptible mice.

mice of either sex with given values of IUdR in the spleen were very close to a theoretical distribution of 56.25% resistant, 37.5% intermediate, and 6.25% susceptible mice. Such a distribution was expected on the assumption that resistance was due to dominant alleles of two independent autosomal genes, and susceptibility to the recessive alleles. If the two genes had additive effects, partial resistance of intermediate segregants was due to dominant alleles at one of the two loci. The expected frequencies of splenic uptake of IUdR in F_2 mice

were also plotted in Fig. 1 by calculating 56.25 and 6.25% of the frequencies observed in resistant and susceptible reference populations, respectively. These percentage values reflect the classical mendelian segregation ratio of 9:6:1 for two independent gene loci. 9 of 16 F_2 segregants possess at least one dominant allele at both loci and 6 of 16 segregants at one locus; 1 of 16 segregants possesses two recessive alleles at both loci.

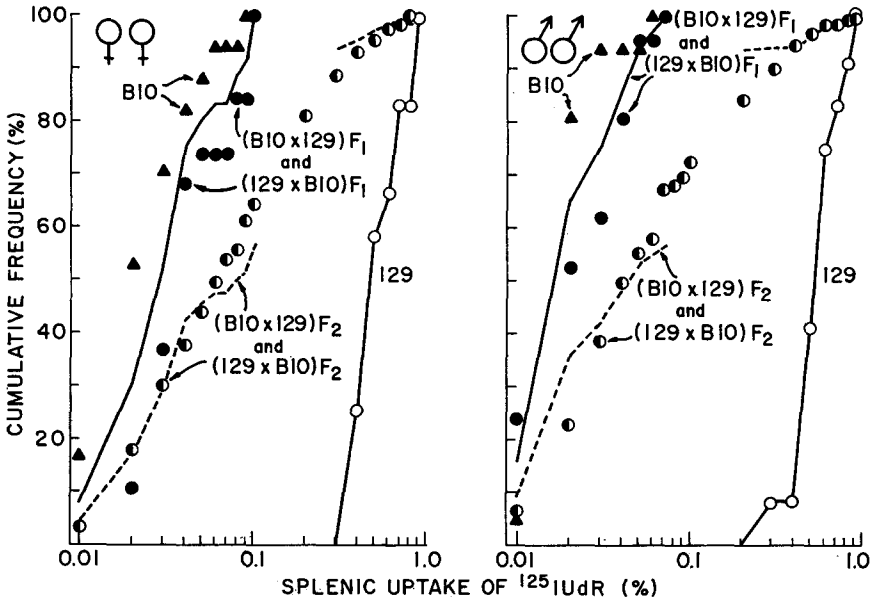

FIG. 1. Cumulative frequencies of splenic uptake values of IUdR in 33 (17 ♀, 16♂) B10 and B10.*tf*,*T* mice, 40 (19 ♀, 21♂) (B10 × 129)F_1 and reciprocal hybrids, 24 (12 ♀, 12♂) 129-strain mice, and 252 (117 ♀, 135♂) F_2 progeny mice. Solid lines represent observed frequencies in reference populations resistant (B10 and F_1 hybrids) or susceptible (129 strain) to DBA/2 marrow cell grafts. The dashed lines represent calculated frequencies for F_2 mice assuming that the segregation ratio for resistant, intermediate, and susceptible F_2 mice was 9:6:1 (i.e., 56.25, 37.5, and 6.25%).

129-strain mice entering the F_1 crosses were white-bellied agouti (A^w/A^w) chinchilla (c^{ch}/c^{ch}) in coat color, and pink-eyed dilute (p/p). B10.*tf*, *T* mice were tufted (*tf*/*tf*) and short-tailed ($T/+$); their coat was nonagouti full color, and their eyes dark. The F_2 mice tested for resistance to DBA/2 marrow grafts were, therefore, segregating for the five marker genes. Segregation data for *tf* and *T*, two mutant alleles of loci lying 7 and 15 crossover units, respectively, from the K end of *H-2* (29), are shown in Table II.

The short-tail phenotype, due to the dominant *T* allele, was expected in one-half of the 252 classified segregants and observed in 118. The tufted-hair phenotype, due to the recessive *tf* allele, was expected in one-fourth of the

segregants, but observed in 42. A nonparental combination of phenotypes specified by the two marker genes (tufted hair, long tail) was noted in eight F_2 mice. The frequency of these crossing-over segregants was compatible with the established map distance of the two loci in linkage group IX (29). Resistance or susceptibility to DBA/2 marrow grafts was not associated with the phenotypes specified by the *tf* and *T* markers. It was, therefore, concluded that none of the major genes regulating allograft rejection lay near the *H-2* region which is

TABLE II

*Independent Segregation of Resistance to DBA/2 Marrow Grafts and Genetic Markers of Linkage Group IX in F_2 Progeny of (B10 × 129)F_1 and Reciprocal Hybrids**

Phenotypes	Genotypes	Resistant (0–0.1% IUdR)		Intermediate‡ (0.11–0.3% IUdR)		Susceptible (0.31–0.9% IUdR)		Total
		♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	
Nontufted, long tail	+/+, +/+ or *tf*/+, +/+	44	54	8	10	7	3	126
Nontufted, short tail	*tf*/+, *T*/+ or +/+, *T*/+§	19	36	8	9	5	7	84
Tufted, short tail	*tf*/*tf*, *T*/+	11	7	9	3	1	3	34
Tufted, long tail	*tf*/*tf*, +/+§	3	1	2	2	0	0	8
All phenotypes		175		51		26		252

* Of 296 F_2 mice born, 44 died before the typing for resistance was completed.

‡ Mice of the parental reference populations grafted with 5×10^5 DBA/2 marrow cells had spleens retaining either less than 0.1% IUdR (resistant) or more than 0.31% (susceptible). The F_2 mice with intermediate values of splenic uptake of IUdR were regarded as a distinct class of segregants rather than as an overlap group.

§ Crossing over between *tf* and *T* loci. All other gene combinations were present in chromosomes of F_1 mice.

seven cross-over units away from *tf*. Had one of the regulator genes been linked with *tf* and *T*, then all resistant segregants (possessing one dominant allele of all regulator genes involved) should have been short-tailed and tufted, except for crossing overs.

Segregation data for the dominant marker gene A^w in linkage group V and for the recessive genes c^{ch} and *p* in linkage group I are shown in Table III. Phenotypes specified by dominant alleles were expected in three-fourths of the F_2 segregants, and those specified by recessive alleles in one-fourth. The frequencies of observed phenotypes were compatible with these expectations. Resistance to DBA/2 marrow grafts was not associated with the nonagouti, full-color, and dark-eye phenotypes, thus excluding that one of the major regulator genes

of graft rejection was closely linked with the marker genes segregating in this cross.

Segregation of Resistance and Susceptibility to B10 Grafts in Backcross Mice

(B10.BR × C3H)F_1 hybrids of both sexes were backcrossed to C3H and the progeny were classified as to resistance or susceptibility. The marker gene *Sl* was brought into the cross either by the F_1 hybrid or by the C3H mice. *Sl*/+ heterozygotes were mated to wild-type+/+ mice to avoid the lethal effect of homozygosity for the *Sl* allele in backcross progeny. A total of 149 (76 females, 73 males) backcross animals were irradiated (850 R) and grafted with 5×10^5 B10 marrow cells from donors of the same sex. Reference populations of 25 resistant B10.BR, 21 resistant (B10.BR × C3H)F_1, and 51 susceptible C3H mice were grafted along with the segregating mice. The cumulative frequencies for given values of splenic uptake of IUdR, determined 5 days after transplantation, are shown in Fig. 2.

TABLE III

*Independent Segregation of Resistance to DBA/2 Marrow Grafts and Genetic Markers of Linkage Groups V and I in F_2 Progeny of (B10 × 129)F_1 and Reciprocal Hybrids**

Phenotypes	Genotypes	Resistant (0–0.1% IUdR)		Intermediate (0.11–0.3% IUdR)		Susceptible (0.31–0.9% IUdR)		Total
		♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	
White-bellied agouti	A^w/A^w and $A^w/+$	56	72	19	16	10	8	181
Nonagouti	+/+	21	26	8	8	3	5	71
Full color	+/+ and $+/c^{ch}$	57	84	24	22	12	9	208
Chinchilla	c^{ch}/c^{ch}	20	14	3	2	1	4	44
Dark eye	+/+ and +/*p*	56	81	21	16	13	8	195
Pink eye dilute	*p*/*p*	20	18	6	8	0	5	57

* Same animals as those typed for tufted hair and short tail (Table II).

In irradiated B10.BR and (B10.BR × C3H)F_1 mice grafted with B10 cells, the splenic uptake of IUdR ranged from 0.01 to 0.04%, and in irradiated C3H mice from 0.21 to 1.0%. Female and male reference populations did not overlap for IUdR uptake, and susceptible mice were separated from resistant mice by a gap. In backcross animals the range of IUdR uptake values was much wider, from 0.01 to 1.0%. As in the preceding intercross, segregants were either like the parental and F_1 reference animals, or intermediate with respect to splenic uptake of IUdR. The observed frequencies of backcross mice with given values of IUdR were close to, but not identical with, a theoretical distribution of 50% resistant, 25% intermediate and 25% susceptible mice. Such a distribution could have resulted from regulation of allograft rejection by two major independent autosomal genes whose dominant alleles were additive in conferring resistance. The results were not compatible with other models, e.g., regulation by one or three independent genes. The percentages used to calculate expected

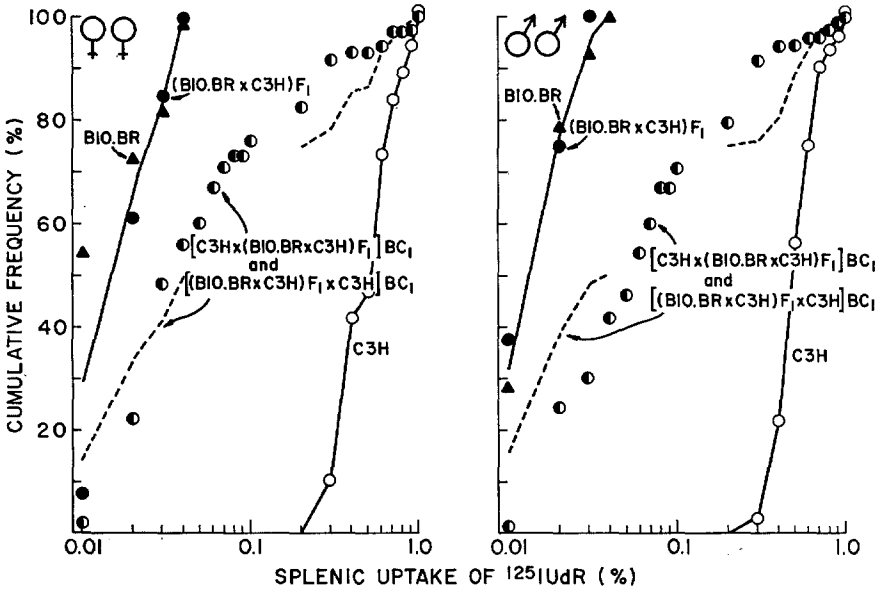

FIG. 2. Cumulative frequencies of splenic uptake values of IUdR in 25 (11 ♀, 14♂) B10.BR mice, 21 (13 ♀, 8♂) (B10.BR × C3H)F_1 hybrids, 51 (19 ♀, 32♂) C3H and C3H.*Sl* mice, and 149 (76 ♀, 73♂) backcross progeny mice. Solid lines represent observed frequencies in reference populations resistant (B10.BR and F_1 hybrids) or susceptible (C3H) to B10 marrow cell grafts. The dashed lines represent calculated frequencies for backcross mice assuming that the segregation ratio for resistant, intermediate, and susceptible mice was 2:1:1 (i.e., 50, 25, and 25%).

TABLE IV

*Independent Segregation of Resistance to B10 Marrow Grafts and the Marker Gene Steel of Linkage Group IV in Progeny of (B10.BR × C3H)F_1 Hybrids Backcrossed to C3H Mice**

Phenotype	Genotype	Resistant 0–0.04% IUdR		Intermediate‡ 0.04–0.2% IUdR		Susceptible 0.21–1.0% IUdR		Total
		♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	
Nondiluted coat color	+/+	18	14	11	15	6	12	76
Diluted coat color	*Sl*/+	25	16	9	13	7	3	73
All phenotypes		73		48		28		149

* Of 181 backcross mice born, 32 died before the typing for resistance was completed.

‡ Mice of the parental reference populations grafted with 5×10^5 B10 marrow cells had spleens retaining either less than 0.04% IUdR (resistant) or more than 0.21% (susceptible). The backcross mice with intermediate values of splenic uptake of IUdR were regarded as a distinct class of segregants rather than as an overlap group.

frequencies of IUdR uptake values in backcross mice reflect the mendelian segregation ratio of 2:1:1 for two independent gene loci.

Segregation data for the dominant marker gene *Sl* in linkage group IV are shown in Table IV. The *Sl*/+ phenotype (diluted coat color) was expected in one-half of the 149 segregants and found in 73. Resistance to B10 marrow grafts was not associated with the wild-type phenotype, thus excluding close linkage of one of the regulator genes with the *Sl* locus.

DISCUSSION

These studies demonstrate the existence of a genetic control for the peculiar mechanism of allograft rejection in heavily irradiated mice given transplants of *H-2*-incompatible marrow. The allograft response does not require induction or proliferation of host lymphoid cells, is thymus-independent, and is effected by relatively radioresistant cells derived from host bone marrow (28). The effector cells seem to be triggered by *H-2* alloantigens of hemopoietic cells but not by the *H-2* antigens of epithelial cells. Control of this rejection process by a small number of genes not linked with *H-2* establishes another major difference with the reactions to *H-2*-incompatible epithelial grafts not influenced by the host's genetic background.

The members of two pairs of mouse strains, B10-129($H\text{-}2^b$) and B10.BR-C3H($H\text{-}2^k$), differed sharply from each other in their ability to reject marrow allografts of given *H-2* type. B10 mice were resistant and 129 mice were susceptible to DBA/2 grafts; B10.BR were resistant to grafts of the congenic strain B10, and C3H mice were susceptible. In each pair the member strains differed at two independent autosomal gene loci regulating marrow graft rejection. The dominant alleles conferred responder status, i.e. the ability to reject, and each locus contributed additively to the strength of the allograft reaction. The recessive alleles conferred nonresponder status, i.e., susceptibility to allografts. Since all mice were transplanted with a relatively small number of cells, only the effects of major regulator genes were detected. The estimates of the number of regulator genes derived from the intercross and backcross described were, therefore, minimum estimates. As segregant mice could not be progeny-tested after irradiation and challenge with allografts, the estimates were also tentative. Likewise, the lack of sex influence on graft rejection could have been the consequence of the small size of allografts; while 5×10^5 donor cells were promptly rejected in these experiments by resistant animals of both sexes, a larger number of cells, just below the number required to override resistance, could have detected sex differences in reactivity. However, it was not practical to progeny-test and graft with graded numbers of cells large populations of segregating mice. An alternative approach is to determine whether mice of a series of congenic lines and of the appropriate background strain are resistant or susceptible. Mice of 10 different lines of the B10.129 series are being challenged with

DBA/2 grafts; each line is congenic with the B10 strain (resistant to DBA/2 grafts) except for a chromosome segment from strain 129 (susceptible to DBA/2 grafts). Preliminary data confirmed that a small number of autosomal genes not linked with *H-2* were additive in regulating marrow graft rejection, but also uncovered a strong sex influence; the resistance of males was two to three times as strong as that of females.

An interesting observation emerging from these and the preceding studies (28) is the similarity of the genetic controls of marrow graft rejection and of antibody responses. The genetic influences regulating the two processes are of the same type in mice, i.e., polygenic with dominant alleles for responsiveness, determinant-specific, and expressed at the level of bone marrow and marrow-derived cells (1, 6, 28, 32). The last two properties of regulator genes suggest that they control early steps in marrow graft rejection and antibody formation, possibly the recognition of *H-2* and other antigens. The cell type which transfers responder and nonresponder status is marrow derived in both systems; thus, marrow precursors of effector cells and of antibody-forming cells are the targets for the genes described in this paper and for *Ir-3* (6, 28, 32). The similarities observed do not prove, however, a common mechanism of gene action, but are compatible with this view. Another significant similarity between *Ir* genes and those regulating graft rejection is the close association with *H* genes (see below).

It has been hypothesized (*a*) that recognition of *H-2* alloantigens of hemopoietic target cells by host effector cells is influenced by the surface patterns specified by so-called "minor" *H* genes; (*b*) that similarity of these patterns on the surface of effector and target cells favors recognition of incongruence at the sites specified by *H-2*; and (*c*) that incongruence of the minor *H* patterns reduces and in extreme cases prevents recognition of nonidentical *H-2* sites (28). Some of the experimental evidence supporting this hypothesis was already described; the study of the B10.129 lines may provide crucial evidence in support of the contention that minor *H* genes were the regulators of marrow graft rejection. Minor *H* loci or closely associated genes could be responsible for the reactivity to DBA/2 grafts in B10 and 129 mice. Transfer of *H* alleles from strain 129 onto the genetic background of strain B10 (31) was sufficient to confer susceptibility to DBA/2 grafts upon mice of at least four B10.129 lines congenic with the resistant B10 strain.[2] The interpretation of these findings is complicated, however, by the fact that some of the congenic lines may carry one or more contaminating genes outside the marked chromosome segment (G. D. Snell, personal communication). The ultimate test as to whether susceptibility to DBA/2 grafts is due to the introduced *H* alleles or to other genes will have to rest on appropriate outcrosses and backcrosses.

None of the major regulators of marrow graft rejection were detectably linked

[2] Cudkowicz, G. Regulatory function of minor *H* loci on rejection of *H-2*-incompatible marrow grafts? In preparation.

to either *H-2* or A^w, thus excluding identity with *Ir-1* and *Ir-2* (1). *Ir-3* has not yet been mapped (1), and it is not known whether it has any effect on reactivity to *H-2*–incompatible marrow grafts. *Ir-4* (8) is of particular interest because it is independent of *H-2* but controls humoral antibody formation to the specificity H-2.2. It is not yet known whether *Ir-4* also influences cell-mediated allograft reactions; its strain distribution makes it an unlikely candidate for one of the genes regulating marrow graft rejection. Since all of the latter genes have yet to be mapped, it is not known whether the regulator genes and alleles distinguishing the two pairs B10-129 and B10.BR-C3H were the same or not. It is anticipated that association of the regulatory function with minor *H* genes will enable fine analysis of the genetic control of specificity of allograft reactions similar to that of antibody responses to synthetic polypeptides (1, 6, 32) and to lysozyme (7). Moreover, it is likely that studies such as these will have implications for clinical bone marrow transplantation, particularly for the choice of the most appropriate donors.

SUMMARY

Transplantation of 5×10^5 DBA/2 ($H\text{-}2^d$) bone marrow cells into irradiated B10 and 129-strain mice (both $H\text{-}2^b$) resulted in graft failure in the first recipient strain and in graft take in the second. Transplantation of B10 ($H\text{-}2^b$) cells into irradiated B10.BR and C3H mice (both $H\text{-}2^k$) also resulted in failure in the congenic B10.BR recipients and take in the C3H mice. Resistance and susceptibility of B10 and 129-strain animals were specific for given *H-2* alleles of donor cells. Transplantation of DBA/2 marrow into (B10 × 129)F_2 mice and of B10 marrow into (B10.BR × C3H)F_1 × C3H backcross mice revealed definite genetic control of the graft-rejection process, presumably at the level of alloantigen recognition. Resistance to allografts, or responder status, was conferred upon segregating mice by dominant alleles of two major independent autosomal loci. The effects of the loci were additive. Conversely, susceptibility to allografts, or nonresponder status, was due to the apparently recessive alleles of both loci. None of the genes was closely linked with the markers *tf* (tufted) and *T* (brachyury) of linkage group IX, A^w (white-bellied agouti) of linkage group V, *Sl* (steel) of linkage group IV, and c^{ch} (chinchilla) and *p* (pink eye, dilute) of linkage group I. There were suggestions, however, that the regulator genes of marrow graft rejection are either non–*H-2* histocompatibility genes or other genetic factors closely linked with them.

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