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)

(Received for publication 14 April 1971)

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

* Research supported by U.S. Public Health Service grant AM-13,969 from the Institute of Arthritis and Metabolic Diseases and by American Cancer Society grant T-476.
resistance and susceptibility to marrow allografts is now described among the progeny of an intercross of (C57BL/10 × 129)F1 mice, and of a backcross of (B10.BR × C3H)F1 mice to C3H parents. The results confirm that differences between the two $H-2^k$ 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, F1, F2, 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 BI0), J. H. Stimpfling, Columbus Hospital, Great Falls, Mont. (B10.A, B10.BR), L. B. Russell, Oak Ridge National Laboratory, Oak Ridge, Tenn. (C3H/He.SI), and T. S. Hauschka, Roswell Park Memorial Institute, Buffalo, N. Y. (C3H/He, DBA/2Ha, and 129/Rt). C3H/He.SI mice of genotype $S/I^+$/ were congenic with C3H/He mice; the former were used for outcrosses with B10.BR and for backcrosses because the coat color of $S/I^+$/ segregants provided a genetic marker of linkage group IV (29).

B10.tf, T mice of genotype $t//f, T^+/-$ (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 ($t//f$) and brachyury ($T$) alleles of the marker stock were transferred onto the B10 genetic background by 10 consecutive backcrosses of $t//f^+/, T^+/+$ mice to B10. Heterozygotes of the last backcross generation were intercrossed and $t//f^+/, T^+/+$ 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 $H-2$ allantigens 2 and 5, and negative for 1, 3, 4, 8, 11, 23 and 31, like B10 erythrocytes. However, the B10 and B10.tf, T lines were congenic for $t//f$, $T$ 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 F2 intercrosses because the tufted hair and short tails of $t//f^+/, T^+/+$ segregants provided genetic markers of linkage group IX (29).

F1 hybrid, F2 intercross, and backcross mice were designated by listing first the female and then the male parental strain, e.g., (B10.BR $♀ \times$ C3H $♂$)F1 $♀ \times$ 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 125I, 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 Diseases, 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^a) 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 host 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^b) grafts.

**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 F2 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 F1 hybrid mice. The observed frequencies of F2

TABLE I

| Donor strain | No. of cells grafted | Strain | Genotype* | No. and sex | % of splenic uptake of IUdR† (geometric mean ± se) |
|--------------|----------------------|--------|-----------|-------------|-----------------------------------------------|
| DBA/2        | 3 × 10^6             | DBA/2  |           | 5           | 0.81 ± 0.08                                   |
|              | 129                  | A^w/A^w |           | 5           | 0.53 ± 0.08                                   |
|              | B10                  | t/t+/T/+|           | 4           | 0.01                                          |
|              | B10 × 129            | t/t+   |           | 3           | 0.01                                          |
|              | 129 × B10            | t/t+   |           | 3           | 0.03                                          |
|              | B10 × 129            | t/t+/, T/+|          | 3           | 0.03                                          |
| B10          | 5 × 10^6             | B10    |           | 6           | 0.46 ± 0.06                                   |
|              | C3H                  |        |           | 4           | 0.50 ± 0.05                                   |
|              | C3H                  | s/l/+   |           | 5           | 0.52 ± 0.04                                   |
|              | B10.BR               |        |           | 5           | 0.02                                          |
|              | B10.BR × C3H         |        |           | 6           | 0.02                                          |
|              | B10.BR × C3H         | s/l/+   |           | 5           | 0.02                                          |
| C3H          | 5 × 10^6             | C3H    |           | 4           | 0.44 ± 0.04                                   |
|              | B10                  |        |           | 9           | 0.32 ± 0.03                                   |
|              | 129                  |         |           | 5           | 0.35 ± 0.02                                   |

* Marker genes to be introduced in F3 intercrosses and backcrosses. +, wild-type allele; A^w, white-bellied agouti; c^eh, chinchilla; p, pink eye; t/t, tufted; T, brachyury; s/l, steel.
† 5 days after irradiation (800 R to inbred mice and 850 R to F1 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 F2 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 F2 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.

129-strain mice entering the F1 crosses were white-bellied agouti (\(A^w/A^w\)) chinchilla (\(c^b/c^b\)) in coat color, and pink-eyed dilute (\(p/p\)). B10, T mice were tufted (\(t/t\)) and short-tailed (\(T/+\)); their coat was nonagouti full color, and their eyes dark. The F2 mice tested for resistance to DBA/2 marrow cell grafts were, therefore, segregating for the five marker genes. Segregation data for \(t/t\) 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 \(t/t\) allele, was expected in one-fourth of the

---

**Fig. 1.** Cumulative frequencies of splenic uptake values of IUdR in 33 (17 B10, 16 129) B10 and B10 x 129 F1 mice, 40 (19 B10, 21 B10 x 129) reciprocal hybrids, 24 (12 B10, 12 129) B10 x 129 F1 progeny mice. Solid lines represent observed frequencies in reference populations resistant (B10 and F1 hybrids) or susceptible (129 strain) to DBA/2 marrow cell grafts. The dashed lines represent calculated frequencies for F2 mice assuming that the segregation ratio for resistant, intermediate, and susceptible F2 mice was 9:6:1 (i.e., 56.25, 37.5, and 6.25%).
segregants, but observed in 42. A nonparental combination of phenotypes specified by the two marker genes (tufted hair, long tail) was noted in eight F2 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

| Phenotypes                  | Genotypes       | Resistant (0-0.1% IUdR) | Intermediate (0.11-0.3% IUdR) | Susceptible (0.31-0.9% IUdR) | Total |
|-----------------------------|-----------------|-------------------------|-------------------------------|-----------------------------|-------|
| Nontufted, long tail        | +/+ or +/+      | 44                      | 54                            | 8                           | 10    |
| Nontufted, short tail       | \( tf/+, +/+ \) | 19                      | 36                            | 8                           | 9     |
| Tufted, short tail          | \( tf/+, T/+ \) | 11                      | 7                             | 9                           | 3     |
| Tufted, long tail           | \( tf/+, +/+ \) | 3                       | 1                             | 2                           | 0     |
| All phenotypes              |                 | 175                     | 51                            | 26                          | 252   |

*Of 296 F2 mice born, 44 died before the typing for resistance was completed.
‡ Mice of the parental reference populations grafted with \( 5 \times 10^5 \) DBA/2 marrow cells had spleens retaining either less than 0.1% IUdR (resistant) or more than 0.31% (susceptible). The F2 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 F1 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 \( e^h \) and \( p \) in linkage group I are shown in Table III. Phenotypes specified by dominant alleles were expected in three-fourths of the F2 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 X C3H)F1 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 F1 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 140 (76 females, 73 males) backcross animals were irradiated (850 R) and grafted with 5 x 10^6 B10 marrow cells from donors of the same sex. Reference populations of 25 resistant B10.BR, 21 resistant (B10.BR X C3H)F1, 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**

| Phenotypes | Genotypes | Resistant (0-0.1% IUdR) | Intermediate (0.11-0.5% IUdR) | Susceptible (0.51-0.9% 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 X C3H)F1 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 F1 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
GENETIC CONTROL OF BONE MARROW GRAFT REJECTION

Fig. 2. Cumulative frequencies of splenic uptake values of IUDR in 25 (11♀, 14♂) B10.BR mice, 21 (13♀, 8♂) (B10.BR × C3H)F1 hybrids, 51 (19♀, 32♂) C3H and C3H.SI mice, and 149 (76♀, 73♂) backcross progeny mice. Solid lines represent observed frequencies in reference populations resistant (B10.BR and F1 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)F1 Hybrids Backcrossed to C3H Mice*

| Phenotype          | Genotype | Resistant | Intermediate | Susceptible | Total |
|--------------------|----------|-----------|--------------|-------------|-------|
|                    |          | 0-0.04%IUDR | 0.04-0.2%IUDR | 0.21-1.0%IUDR |       |
| Nondiluted coat color | +/+     | 18        | 14           | 11          | 15    | 6     | 12    | 76    |
| Diluted coat color  | S+/+    | 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 × 10⁶ 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-2k*) and B10.BR-C3H(*H-2T*), 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 $\times$ 10^4 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 hematopoietic 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. 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

---

Cudkowicz, G. Regulatory function of minor H loci on rejection of H-2-incompatible marrow grafts? In preparation.
to either \( H-2 \) or \( A^n \), 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 not 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 \( 5 \times 10^5 \) DBA/2 \( (H-2^a) \) bone marrow cells into irradiated B10 and 129-strain mice (both \( H-2^a \)) resulted in graft failure in the first recipient strain and in graft take in the second. Transplantation of B10 \( (H-2^b) \) cells into irradiated B10.BR and C3H mice (both \( H-2^k \)) 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 X 129)\( F_1 \) mice and of B10 marrow into (B10.BR X C3H)\( F_1 \) X 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, \( A^n \) (white-bellied agouti) of linkage group V, \( St \) (steel) of linkage group IV, and \( c^h \) (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.

The author is grateful to Doctors L. C. Dunn, T. S. Hauschka, G. D. Snell, J. H. Stimpfling, and L. B. Russell for kindly providing breeding pairs of the mouse strains used in this study. The help of Dr. E. Lotzová in serotyping B10\( tf \), \( T \) mice and the generous supply of antisera by the Transplantation Immunology Branch, National Institute of Allergy and Infectious Diseases, are also acknowledged.
BIBLIOGRAPHY

1. McDevitt, H. O., and B. Benacerraf. 1969. Genetic control of specific immune responses. *Adv. Immunol.* 11:31.

2. Rathbun, W. E., and W. H. Hildemann. 1970. Genetic control of the antibody response to simple haptens in congenic strains of mice. *J. Immunol.* 105:98.

3. Vaz, N. M., and B. B. Levine. 1970. Immune responses of inbred mice to repeated low doses of antigen: relationship to histocompatibility (H-2) type. *Science (Washington).* 168:852.

4. Martin, W. J., L. Ellman, I. Green, and B. Benacerraf. 1970. Histocompatibility type and immune responsiveness in random bred Hartley strain guinea pigs. *J. Exp. Med.* 132:1259.

5. Biozzi, G., R. Asofsky, R. Lieberman, C. Stiffel, D. Mouton, and B. Benacerraf. 1970. Serum concentration and allotypes of immunoglobulins in two lines of mice genetically selected for “high” or “low” antibody synthesis. *J. Exp. Med.* 132:752.

6. Mozes, E., G. M. Shearer, M. Sela, and W. Braun. 1970. Conversion with polynucleotides of a genetically controlled low immune response to a high response in mice immunized with a synthetic polypeptide antigen. In Symposium on Biological Effects of Polynucleotides. R. F. Beers and W. Braun, editors. Springer-Verlag Inc., New York. 162.

7. Mozes, E., E. Maron, R. Arnon, and M. Sela. 1971. Strain-dependent differences in the specificity of antibody responses toward lysozyme. *J. Immunol.* 106: 862.

8. Lilly, F., J. S. Jacoby, and R. C. Coley. 1971. Immunologic unresponsiveness to the H-2.2 antigen. In Immunogenetics of the H-2 System. M. Vojtíšková and A. Lengerová, editors. S. Karger, AG, Basel, Switzerland. In press.

9. Lilly, F. 1971. H-2, membranes and viral leukemogenesis. In Cellular Interactions in the Immune Response. S. Cohen, G. Cudkowicz, and R. T. McCluskey, editors. S. Karger, AG, Basel, Switzerland. 103.

10. Tennant, J. R., and G. D. Snell. 1968. The H-2 locus and viral leukemogenesis as studied in congenic strains of mice. *J. Nat. Cancer Inst.* 41:597.

11. Lilly, F. 1970. Fv-2: identification and location of a second gene governing spleen focus response to Friend leukemia virus in mice. *J. Nat. Cancer Inst.* 46:163.

12. Steeves, R. A., M. Bennett, E. A. Mirand, and G. Cudkowicz. 1968. Genetic control by the W locus of susceptibility to (Friend) spleen focus-forming virus. *Nature (London).* 218:372.

13. Bennett, M., R. A. Steeves, G. Cudkowicz, E. A. Mirand, and L. B. Russell. 1968. Mutant Sl alleles of mice affect susceptibility to Friend spleen focus-forming virus. *Science (Washington).* 162:564.

14. Pincus, T., J. W. Hartley, and W. P. Rowe. 1971. A major genetic locus affecting resistance to infection with murine leukemia viruses. I. Tissue culture studies of naturally occurring viruses. *J. Exp. Med.* 133:1219.

15. Pincus, T., W. P. Rowe, and F. Lilly. 1971. A major genetic locus affecting resistance to infection with murine leukemia viruses. II. Apparent identity to a major locus described for resistance to Friend murine leukemia virus. *J. Exp Med.* 133:1234.
16. Snell, G. D. 1968. The H-2 locus of the mouse: observations and speculations concerning its comparative genetics and its polymorphism. *Folia Biol. (Praha).* 14:335.

17. Burnet, F. M. 1970. A certain symmetry: histocompatibility antigens compared with immunocyte receptors. *Nature (London).* 225:123.

18. Ellman, L., I. Green, and W. J. Martin. 1970. Histocompatibility genes, immune responsiveness, and leukemia. *Lancet.* 1:1104.

19. Hauschka, T. S., S. T. Grinnell, M. Meagher, and D. B. Amos. 1959. Sex-linked incompatibility of male skin and primary tumors transplanted to isologous female mice. In Genetics and Cancer. University of Texas Press, Austin, Texas. 271.

20. Hauschka, T. S., and B. A. Holdridge. 1962. A cytogenetic approach to the Y-linked histocompatibility antigen of mice. *Ann. N. Y. Acad. Sci.* 101:12.

21. Silvers, W. K., and R. E. Billingham. 1967. Genetic background and expressivity of histocompatibility genes. *Science (Washington).* 158:118.

22. Silvers, W. K., R. E. Billingham, and B. H. Sanford. 1968. The H-Y transplantation antigen: a Y-linked or sex-influenced factor? *Nature (London).* 220:401.

23. Klein, E., and O. Linder. 1961. Factorial analysis of the reactivity of C57BL females against isologous male skin grafts. *Transplanta. Bull.* 7:457.

24. Gasser, D. L., and W. K. Silvers. 1971. Genetic control of the immune response in mice. III. An association between H-2 type and reaction to H-Y. *J. Immunol.* 106:875.

25. Bailey, D. W., and J. Hoste. 1971. A gene governing the female immune response to the male antigen in mice. *Transplantation.* 11:404.

26. Bailey, D. W. 1971. Allelic forms of a gene controlling the female immune response to the male antigen in mice. *Transplantation.* 11:426.

27. Cudkowicz, G. 1965. Hybrid resistance to parental hemopoietic cell grafts: implications for bone marrow chimeras. In La greffe des cellules hématoïdiennes allogéniques. Centre National de la Recherche Scientifique, Paris, France. 207.

28. Cudkowicz, G., and M. Bennett. 1971. Peculiar immunobiology of bone marrow allografts. I. Graft rejection by irradiated responder mice. *J. Exp. Med.* 134:83.

29. Green, M. C. 1966. Mutant genes and linkages. In Biology of the Laboratory Mouse. E. L. Green, editor. McGraw-Hill Book Company, New York. 87.

30. Bennett, M., G. Cudkowicz, R. S. Foster, Jr., and D. Metcalf. 1968. Hemopoietic progenitor cells of W anemic mice studied in vivo and in vitro. *J. Cell. Physiol.* 71:211.

31. Graff, R. J., and G. D. Snell. 1969. Histocompatibility genes of mice. IX. The distribution of the alleles of the non-H-2 histocompatibility loci. *Transplantation.* 8:861.

32. Mozes, E., and G. M. Shearer. 1971. Contribution of bone marrow cells and lack of expression of thymocytes in genetic controls of immune responses for two immunopotent regions within poly-(phe, glu)-poly-pro-poly-lys in inbred mouse strains. *J. Exp. Med.* 134:141.