PECULIAR IMMUNOBIOLOGY OF BONE MARROW ALLOGRAFTS

II. Rejection of Parental Grafts by Resistant F_1 Hybrid Mice*

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Mouse bone marrow cells transplanted across the major histocompatibility barrier fail to grow in given strain combinations even after exposure of prospective recipients to lethal doses of total body irradiation (1). The graft failures are presumably due to host reactivity against *Histocompatibility-2 (H-2)*[1] alloantigens which persists after irradiation and does not require presensitization or proliferation of host lymphoid cells. Other properties of this unusual allograft reactivity are its late maturation in infant mice at 3 wk of age, its apparent independence of thymic influence, and its regulation by *immune response* genes not linked to the *H-2* locus (1, 2).

Cells of the bone marrow and other myelopoietic and lymphopoietic organs of inbred mice also fail to grow under conditions in which epithelial grafts are accepted, i.e., upon transplantation from parental-strain donors into irradiated F_1 hybrid recipients (3–12). In most strain combinations, the resistance of F_1 hybrids is attributable to heterozygosity at a locus closely linked to, or part of, the D end of *H-2*, designated *Hybrid-histocompatibility-1* or *Hh-1* (8–10, 13, 14). The reactivity of the F_1 mice is directed against cells bearing the products of homozygous *Hh-1* alleles. Thus, the genetic determination of incompatibility for bone marrow grafts could be different for parent-to-F_1 and allogeneic cell transfers, but the immunobiology of graft rejection may still be the same in the two systems. The experiments described below address themselves to this question; the results obtained indicate that hybrid resistance to marrow grafts of

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[1] *Abbreviations used in paper*: CY, cyclophosphamide; *H-2*, *Histocompatibility-2*, *Hh-1*, *Hybrid-histocompatibility-1*; IUdR, 5-iodo-2′-deoxyuridine.

inbred parental donors homozygous for the *Hh-1*^{a} allele (9, 10) is indeed due to a rejection process resembling very closely that previously described for resistance of inbred mice to allogeneic marrow cells. It could not be established, however, whether the genetic determination of incompatibility is different or not.

Materials and Methods

Mice.—Most inbred and F_1 animals were raised in our animal colony and were derived from pedigreed breeders supplied by G. D. Snell, Jackson Laboratory, Bar Harbor, Maine, (C57BL/10ScSn [abbreviated B10], B10.D2-O, B10.D2-N, A.BY, C3H.SW, D1.LP); E. S. Russell, Jackson Laboratory (WB/Re); J. H. Stimpfling, Columbus Hospital, Great Falls, Mont. (B10.A[2R], B10.A); and T. S. Hauschka, Roswell Park Memorial Institute, Buffalo, N. Y. (129/Rr, C3H/He, C57BL/Ha, DBA/2Ha). Mice of the strains DBA/1 and (C3H/He × C57BL/Ha)F_1 were obtained from the West Seneca Animal Production Unit of Roswell Park Memorial Institute; LP/J from the Jackson Laboratory; C57BL/Cum and (C3H/Anf × C57BL/Cum)F_1 from Cumberland View Farms, Clinton, Tenn.; and NZB from the Animal Production Center, National Cancer Institute, Bethesda, Md. Mice of both sexes, 10–15-wk old, were used in most experiments. F_1 hybrids were designated by listing first the female and then the male parental strain.

Irradiation.—Mice to be grafted with marrow cells were exposed to total body X-irradiation as previously described (15).

Immunization.—Mice were given suspensions of 10^7 viable spleen cells in 0.3 ml of Eagle's medium by intraperitoneal injection. All animals were immunized twice at 7-day intervals and used as marrow graft recipients 4–7 days after the last injection. Spleen cell donors and mice to be immunized differed for alloantigens specified by the *H-2* locus.

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 (1, 15). 4 or 5 days later, the DNA precursor 5-iodo-2′-deoxyuridine (IUdR) labeled with radioactive ^{131}I or ^{125}I (Amersham-Searle Corporation, Arlington Heights, Ill.) was used to assess DNA synthesis by donor-derived cells in recipient spleens, as previously described (15). The values of IUdR uptake in this organ were determined by crystal scintillation counting and expressed as per cent of injected radioactivity retained. Geometric means ± standard errors were calculated from uptake values of individual spleens belonging to groups of mice given identical treatment. Negative controls were irradiated mice not injected with marrow cells; the uptake of IUdR in such spleens was not greater than 0.05%. Positive controls were irradiated mice grafted with 0.5–1 × 10^6 syngeneic cells; the splenic uptake values of IUdR were 0.3–1.0%.

Proliferation of grafted cells was also evaluated qualitatively by gross inspection of recipient spleens fixed in Bouin's fluid. Since fixation did not remove radioactive IUdR, both methods could be applied to the same spleens. In mice susceptible to marrow grafts, the spleens were nodular 5 days after transplantation of 10^6 cells, but not so in resistant mice or in radiation controls (Fig. 1). The nodular areas were lighter in color than the surrounding splenic tissue and visible by the unaided eye; in histological preparations such areas appeared to be very active centers of hemopoietic repopulation (15). Classification of mice as resistant or susceptible by the inspection and the IUdR uptake methods were always in agreement.

Calculation of the Growth Index.—For the convenience of data presentation and of comparisons between separate experiments, results of bone marrow transplants in syngeneic and F_1 hybrid mice were expressed in relative terms. In each experiment, a given number of donor cells was transplanted into syngeneic and F_1 hybrid mice of the same sex and age. Assuming that the syngeneic hosts provided the optimal conditions for the proliferation of grafted cells,

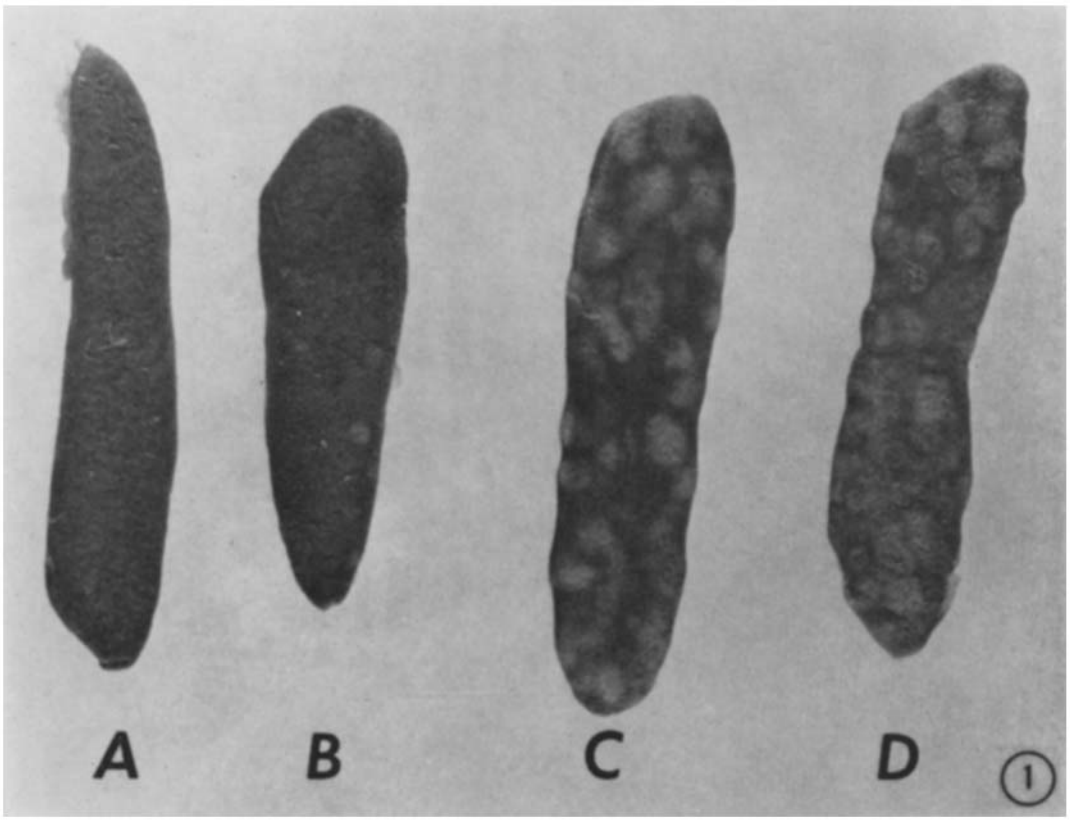

FIG. 1. Representative spleens of (C3H/He × C57BL/Ha)F_1 mice exposed to 900 R of X-rays and injected with saline (radiation controls) (*A*), 10^6 C57BL/Ha marrow cells (*B*), 10^6 C3H/He marrow cells (*C*), or 10^6 syngeneic marrow cells (*D*). The mice were injected with IUdR 5 days after marrow grafting, and the spleens were removed and fixed in Bouin's fluid 17 hr later. The nodules grossly apparent represent foci of hemopoiesis, which can be readily identified microscopically. Note the similarity between *A* and *B* (0.01–0.05% uptake of IUdR) and between *C* and *D* (0.3–1.0% uptake of IUdR). × 6.5.

the growth index in nonsyngeneic hosts was calculated according to the equation:

$$\text{Growth index} = \frac{\text{mean uptake of IUdR in spleens of } F_1 \text{ mice}}{\text{mean uptake of IUdR in spleens of syngeneic mice}} \times 100.$$

RESULTS

Maturation of Hybrid Resistance in Infant Mice.—Two strains of F_1 hybrids were chosen to study the age dependence of hybrid resistance (Fig. 2). The inbred parents of F_1 mice were congenic-resistant pairs, i.e., mice differing from each other by a short chromosome segment which included either the entire

H-2 region (B10 and B10.D2-O) or its D end only (B10.A[2R] and B10.A) (16). The hybrids were, therefore, homozygotes for most of their genome, but heterozygotes for *H-2* or for the D subregion of *H-2*, the one which includes *Hh-1* (9, 10). 0.5–1 × 10^6 bone marrow cells of the parental donors B10 and B10.A(2R) of genotype *Hh-1*^{a}/*Hh-1*^{a} were transplanted into syngeneic and F_1 mice of both sexes a few hours after exposure of the latter to 700 R of X-rays. Splenic uptake of IUdR was determined 5 days later. By repeating the experiments several times with male or female donors and with recipients of varying ages at the time of cell injection, we were able to test hybrids 13–71 days old

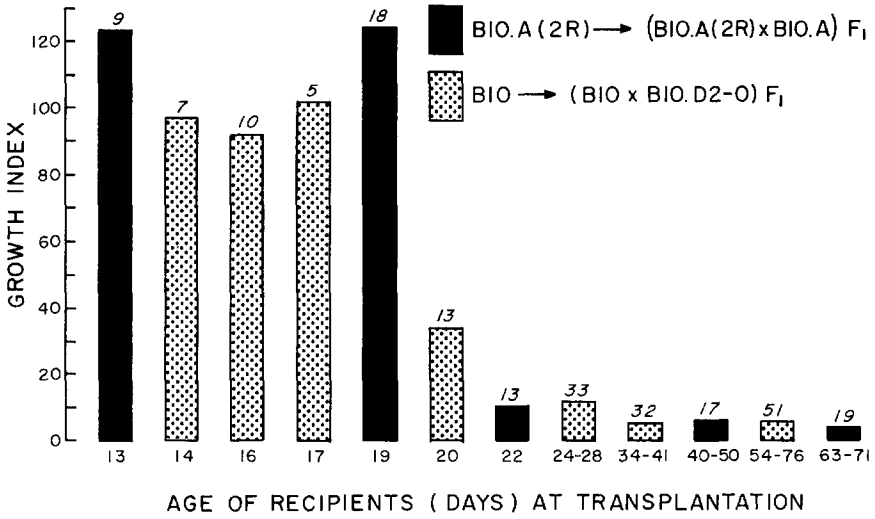

FIG. 2. Maturation of hybrid resistance. Growth indices of parental marrow cells (0.5–1 × 10^6/mouse) 5 days after transplantation into irradiated (700 R) infant and young F_1 hybrid recipients. The numbers of mice tested are indicated above the bars.

and to vary the sexes of donors and recipients in all possible combinations. Suckling mice less than 21 days old and mice of weaning age (21 days) were left with their mothers until completion of the experiments. As the sexes of donors and recipients did not influence the results, data from all mice of given ages were pooled to calculate growth indices. The values of the indices were 90–123 in 13, 14, 16, 17, and 19 days old F_1 mice since IUdR uptake values were about the same in syngeneic and F_1 hosts. All of the 49 suckling hybrids tested (13–19 days) were susceptible to parental marrow grafts and donor cells grew unimpaired. The growth indices were considerably lower (less than 15) between 22 and 71 days of age, and intermediate in 20-day old mice. Thus, the young hybrids became resistant to parental grafts after the 3rd wk of life, the same age at which young inbred mice become resistant to allogeneic marrow grafts (1).

Both kinds of resistance appeared to be due to host function(s) which mature at the same rate in infant mice.

Radiation Sensitivity of Hybrid Resistance.—To determine whether or not hybrid resistance was entirely insensitive to the effects of total body irradiation, the following experiment was set up. Parental grafts of 10^6, 5×10^6, and 10^7 marrow cells were given to adult resistant hybrids of the strains indicated in Fig. 3 a few hours after graded exposures to X-rays in the range of 600–1000 R. Splenic uptakes of IUdR were measured 5 days later. The values of isotope retention in radiation control spleens (i.e. spleens of irradiated mice not in-

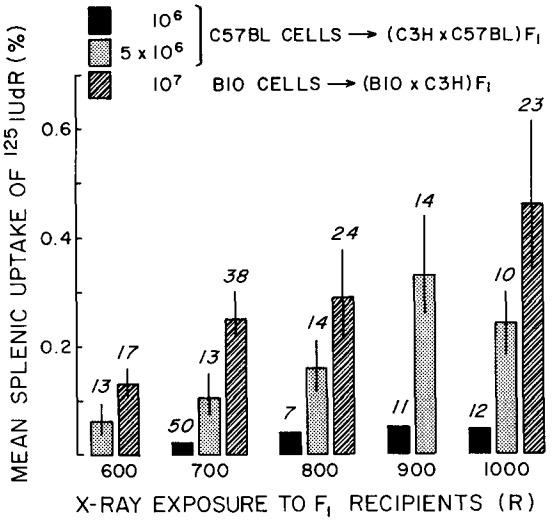

FIG. 3. Radiation sensitivity of hybrid resistance. Mean uptake of IUdR and 95% confidence intervals in spleens of F_1 hybrid mice 5 days after graded exposures of X-rays (600–1000 R) and transplantation of graded numbers of parental marrow cells (1–10 $\times$ 10^6/mouse). (C3H/Anf $\times$ C57BL/Cum)F_1 and (B10 $\times$ C3H/He)F_1 sublines were used. The numbers of mice tested are indicated above the bars.

jected with cells) were subtracted from those of the corresponding experimental spleens to obtain the net values shown in Fig. 3. Growth indices were not calculated because the values of IUdR uptake promoted by 5×10^6 and 10^7 parental cells in spleens of syngeneic hosts are outside the linear portion of the dose-response curve (8, 14, 15).

Grafts of 10^6 cells failed in all groups of F_1 recipients, regardless of the radiation exposures. Grafts of 5×10^6 and 10^7 cells promoted IUdR uptake in recipient spleens, thus overriding hybrid resistance. With increasing doses of X-rays to F_1 mice, the values of IUdR uptake increased, as if resistance was progressively weakened. However, even the largest X-ray exposures did not fully abrogate resistance. Grafts of 10^6 parental cells promoted splenic uptakes of

IUdR of 0.60–1.00% in syngeneic hosts, values greater than those promoted by 5×10^6 or 10^7 cells in F_1 hybrids given 900–1000 R of X-rays.

The refractoriness of hybrid resistance to acute irradiation could best be explained in terms of a host–anti-graft reaction mediated by mature non-dividing cells. However, such cells could belong to a cell-renewal system. This possibility was tested by exposing resistant hybrids and parental-strain mice to a first sublethal dose of radiation (500 R of X-rays) to inhibit proliferation of precursors of effector cells. At variable intervals the mice were exposed to a second lethal dose of X-rays (800 R to parental-strain and 900 R to F_1 mice)

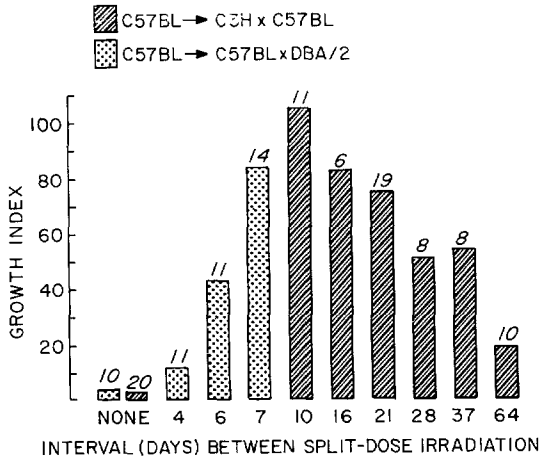

FIG. 4. Weakening of hybrid resistance by split-dose irradiation. Growth indices of parental marrow cells ($0.5–1 \times 10^6$/mouse) 5 days after transplantation into F_1 hybrid recipients irradiated twice (500 + 900 R). The intervals between the first and second exposure varied from 4 to 64 days. (C3H/He × C57BL/Ha)F_1 and (C57BL/Ha × DBA/2Ha)F_1 sublines were used. The numbers of mice tested are indicated above the bars.

and grafted with 0.5 or 1×10^6 parental marrow cells. The results are shown in terms of growth indices in Fig. 4.

Preirradiation of F_1 mice resulted in weakening of resistance not before day 6, and in maximal abrogation at day 10. The preirradiated hybrids remained partially susceptible until day 37 but were resistant again by day 64. Under our conditions, the preirradiation had no detectable effect on the growth of parental cells in syngeneic recipients, as in previous experiments (1). The data suggested that host cells responsible for hybrid resistance belonged to a renewal system whose depletion by 500 R of X-rays became detectable within 1 wk in (C57BL × DBA/2)F_1 mice. Since preirradiated hybrids were still resistant at 4 days, the life-span of the effector cells must have been several days long. The late reacquisition of hybrid resistance implied that reconstitution of the

effector cell population occurred at a slow rate. The proliferative ability of both the stem cells and the more immediate precursors of effector cells was presumably impaired by total body irradiation.

Abrogation of Hybrid Resistance by Cyclophosphamide.—Because of the late maturation of hybrid resistance in infants and the weakening of resistance by split-dose irradiation in adults, it is possible that effector cells are sensitive to the cytocidal effect of cyclophosphamide (CY). The drug (Cytoxan, Mead Johnson and Co., Evansville, Ind.) was injected intraperitoneally, 300 mg/kg of body weight, at varying intervals before irradiation (800 and 900 R to parental-strain

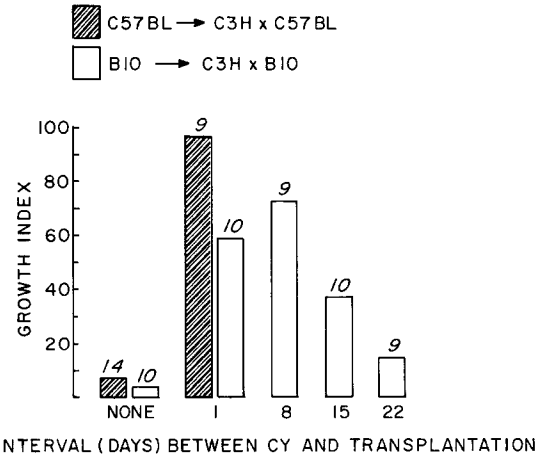

FIG. 5. Weakening of hybrid resistance by cyclophosphamide. Growth indices of parental marrow cells (1.2×10^6/mouse) 4 days after transplantation into irradiated (900 R) F_1 hybrid recipients which had been injected with cyclophosphamide (300 mg/kg intraperitoneally) 1–22 days previously. (C3H/He × C57BL/Ha)F_1 and (C3H/He × B10)F_1 sublines were used. The numbers of mice tested are indicated above the bars.

and F_1 mice, respectively) and transplantation of 1.2×10^6 parental marrow cells. Repopulation of syngeneic and F_1 recipient spleens was assessed 4 days after cell injections and growth indices were calculated. To verify that CY did not enhance background values of IUdR uptake in irradiated mice, control mice were treated with CY, irradiated, but not injected with cells in each experiment. The data are shown in Fig. 5.

Hybrid resistance was either abrogated or weakened 1 day after administration of CY and remained weakened until day 15. The hybrids treated with CY reacquired resistance by day 22. CY must have acted directly upon the effector cells of resistance because its effect was prompt. Most likely, the precursors of effector cells were also affected since partial susceptibility induced by CY lasted 3 wk.

Weakening of Hybrid Resistance by Corynebacterium parvum.—Since *C. parvum* weakened the resistance of inbred mice to allogeneic marrow grafts (1), it was of interest to also determine its effect on hybrid resistance. Heat-killed cultures of *C. parvum* (obtained through the courtesy of Dr. G. Biozzi) were suspended in saline, and 0.5 mg (dry weight) of whole bacteria were injected intravenously into prospective F_1 and syngeneic recipients of parental marrow grafts. After 4–21 days, the mice were irradiated (800–900 R) and grafted with 0.5–1 $\times$ 10^6 marrow cells. Repopulation of recipient spleens was assessed 5 days later and growth indices were calculated. In each experiment, control mice were treated with *C. parvum*, irradiated, but not injected with cells, to verify that the

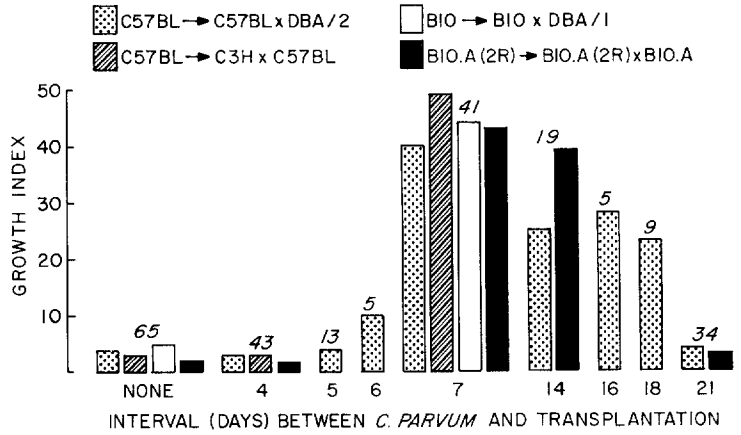

FIG. 6. Weakening of hybrid resistance by *Corynebacterium parvum*. Growth indices of parental marrow cells (0.5–1 $\times$ 10^6 cells/mouse), 5 days after transplantation into irradiated (900 R) F_1 hybrid recipients which had been injected with heat-killed bacteria (0.5 mg, dry weight, intravenously) 4–21 days previously. (C57BL/Ha $\times$ DBA/2Ha)F_1 and (C3H/He $\times$ C57BL/Ha)F_1 sublines were used. The total numbers of mice tested are indicated above the bars.

bacterium did not enhance background values of IUdR uptake in irradiated mice. The data are shown in Fig. 6.

Hybrid resistance was not weakened until day 7 after treatment with *C. parvum*, but remained weakened through day 18. The hybrids injected with *C. parvum* reacquired resistance by day 21. As with radiation, the effector cells were not directly inactivated by *C. parvum*, since there was a lag period of 7 days duration. However, the precursors of effector cells must have been influenced by the dead bacterium since recovery of resistance required a period of 3 wk. In view of the intense lymphoid cell proliferation induced by *C. parvum* (17, 18), it is possible that precursors common to the effector cells of hybrid resistance and to other cell types were temporarily forced to differentiate along pathways leading to the production of different populations of mature cells.

Time of Parental Graft Rejection by Irradiated F_1 Hybrids.—The failure of transplanted parental marrow cells to colonize the spleens of irradiated F_1 recipients was presumably due to the killing of hemopoietic stem cells of donor origin by the host effector cells. Another possibility is that the F_1 environment either did not stimulate, or positively inhibited the differentiation of stem cells without killing them. The latter hypothesis would require that pretreatment of F_1 hybrids with X-rays, CY, and *C. parvum* modified the interactions of a non-immunological nature between host and donor cells postulated by several

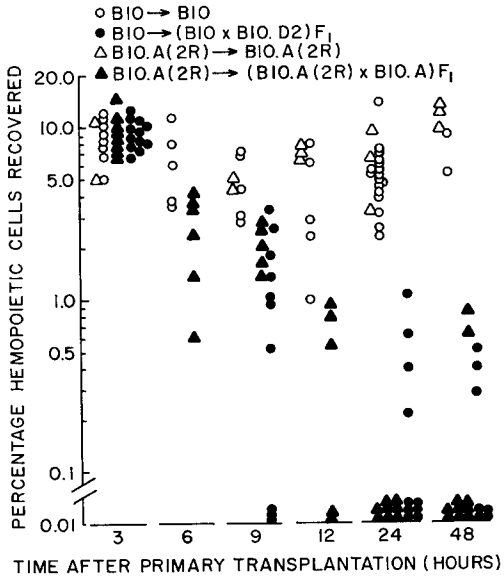

FIG. 7. Recovery of retransplantable parental hemopoietic cells from the spleens of recipient mice (syngeneic and F_1 hybrid) at various times after transplantation of 2.5×10^6 marrow cells. Points represent the percentages of cells recovered from the whole spleens of individual primary recipients, as estimated by retransplantation into secondary parental-strain recipients. The 100% value was estimated from primary syngeneic recipients given 2.5×10^5 marrow cells.

experimental hematologists (19–21). The question was examined with a retransplantation experiment.

Spleens of F_1 and parental-strain mice were sampled 3–48 hr after irradiation (800–900 R) and transplantation of 2.5×10^6 parental marrow cells. The cells of the spleen of each primary recipient were suspended and retransplanted into one irradiated (800 R) secondary recipient which was syngeneic with the original marrow donors. The secondary recipients were previously immunized against antigens of the second parental strain of the primary F_1 recipients, i.e., $P_1 \rightarrow (P_1 \times P_2)F_1 \rightarrow P_1{}^{*P_2}$, where the asterisk denotes immunization. The strain combinations used are indicated in Fig. 7; transplants were made in female and

male mice, but the sexes of donors and recipients were always matched. Proliferation of the original donors' hemopoietic cells was assessed in secondary recipients by the IUdR uptake method 5 days after retransplantation. For each experiment, a group of syngeneic mice was injected with a smaller inoculum of 2.5×10^5 parental cells (instead of 2.5×10^6) and assayed 5 days later for splenic uptake of IUdR. The value so obtained was multiplied by 10 to estimate the IUdR uptake expected in secondary recipients if 100% of the injected hemopoietic stem cells were recovered from primary recipient spleens. The percentages of cells actually recovered from each of the 55 syngeneic and 82 F_1 hybrid primary recipients are shown in Fig. 7.

During the first 3 hr after transplantation of parental marrow cells, recovery was the same in the two groups of susceptible (syngeneic) and resistant F_1 mice. At 6 hr the fraction of recovered cells was slightly lower in F_1 hybrids. At later intervals the difference between the two groups became greater because of expansion of hemopoietic cells in the spleens of syngeneic hosts and loss of cells in the spleens of F_1 hosts. Parental stem cells did not replicate (Fig. 7) nor differentiate beyond 24–48 hr. The data strongly suggest that the cells were rejected beginning 9–12 hr after transplantation.

Specific Suppression of Hybrid Resistance.—(C3H/Anf × C57BL/Cum)F_1 mice are resistant to marrow grafts from C57BL and other inbred-strain donors sharing the *H-2*^{b} and the closely linked *Hh-1*^{a} alleles (9, 10). The resistance of such hybrids to C57BL can be abrogated by four injections of C57BL spleen cells given at weekly intervals, but not by injections of spleen cells of the other parental strain, i.e., C3H (22). The hybrids so treated were not chimeras for hemopoietic cells as judged by hemoglobin markers and by retransplantation of the hybrids' spleen cells (22). A large number of (C3H × C57BL)F_1 mice were now rendered susceptible to C57BL marrow grafts by four weekly intraperitoneal injections of 2×10^7 C57BL spleen cells. The abrogation of hybrid resistance and the extent of its specificity were tested 1 wk after the last spleen cell injection in two ways. Firstly, groups of hybrids pretreated with spleen cells of the second parental strain or of a third unrelated strain were compared with hybrids injected with C57BL spleen cells. Secondly, groups of pretreated hybrids were test grafted with bone marrow cells of a variety of donor strains which were either *H-2*^{b}*–Hh-1*^{a}, like C57BL (9, 10), or of a different type. The test grafts were 10^6 nucleated cells injected into irradiated (900 R) F_1 mice. Splenic uptake of IUdR was measured 5 days later and the mean values are reported in Table I.

Hybrid resistance to C57BL cells was abrogated by pretreatment with C57BL but not with C3H spleen cells, as reported earlier (22). Resistance to marrow cells of five other mouse strains, unrelated by ancestry to C57BL (23) but sharing the *H-2*^{b}*–Hh-1*^{a} alleles, was also fully abrogated by pretreatment of the hybrids with C57BL cells. C3H or third party (DBA/2) spleen cells had no

effect on resistance to marrow grafts of the same donor strains. The resistance of (C3H × C57BL)F_1 mice to cells of four allogeneic donor strains of different *H-2* and *Hh-1* type (13) was neither abrogated nor weakened by the C57BL, C3H, or DBA/2 spleen cell injections. It is noteworthy that F_1 hybrids were able to resist allogeneic grafts after four injections of parental spleen cells. Thus, the mild graft-*versus*-host reaction possibly elicited by the parental cells did not detectably impair the overall reactivity against allografts. The same could

TABLE I

*Proliferation of Bone Marrow Grafts of Inbred-Strain Donors in Irradiated (C3H × C57BL)F_1 Hybrid Recipients Pretreated with Parental or Allogeneic Spleen Cells**

Marrow cell donors		Splenic uptake of IUdR in F_1 recipients pretreated with spleen cells of the following strains‡		
Strain	*H-2* type	None	C3H	C57BL
C57BL/Cum	*b*	0.03	0.03	0.79 ± 0.05
C57BL/Ha	*b*	0.04	0.02§	0.56 ± 0.06
LP	*b*	0.09	0.05	0.65 ± 0.08
129	*b*	0.04	0.03	0.80 ± 0.06
A.BY	*b*	0.05	0.06	0.58 ± 0.05
C3H.SW	*b*	0.07	0.05	0.68 ± 0.07
DL.LP	*b*	0.04	0.04	0.73 ± 0.06
B10.A	*a*	0.04	0.02§	0.05
DBA/2	*d*	0.03	0.02	0.05
B10.D2	*d*	0.03	—	0.03
NZB	*d*	0.02	—	0.02
WB	*ja*	0.02	—	0.03

* 5 days after irradiation (900 R) and transplantation of 10^6 cells. Radiation control values of splenic uptake of IUdR ranged from 0.01 to 0.05%; such values were subtracted from those of experimental animals which were in excess of 0.1%. Results are given as geometric means ± standard errors for groups of 7–10 mice. (C3H/Anf × C57BL/Cum)F_1 and (C3H/He × C57BL/Ha)F_1 sublines were used.

‡ Four intraperitoneal injections of 2×10^7 spleen cells each at weekly intervals. Irradiation and bone marrow transplantation 1 wk after the last injection.

§ Pretreatment with DBA/2Ha instead of C3H spleen cells.

be said for the competence of hybrids to resist parental C57BL grafts after the injections of allogeneic DBA/2 or parental C3H spleen cells.

DISCUSSION

Hybrid resistance meets the criteria of a transplantation reaction for the following reasons: (*a*) adult F_1 mice respond to parental hemopoietic grafts by an immunogenetically specific reaction which is destructive for the grafted cells; (*b*) the hybrids' response can be abrogated by both nonspecific and immunogenetically specific suppressive treatments; (*c*) the physiologic parameters of

the hybrids' response are indistinguishable from those of mice grafted with allogeneic (*H-2*–incompatible) instead of parental-strain marrow cells. However, most of the properties of resistance to hemopoietic grafts differ from the conventional transplantation reactions to epithelial cells. The relative insensitivity to acute total body irradiation and the age of maturation of resistance, the rapid onset and completion of marrow graft rejection, the restriction of hybrid resistance to hemopoietic cells, and the incompatibility of F_1 heterozygotes (at a particular gene locus) for homozygous cells are the major distinguishing features.

Effector cells of hybrid resistance reach a critical population size and/or become functional after the 3rd wk of age. Such effector cells do not need to proliferate since rejection is detectable 9–48 hr after acute irradiation with 900 R of X-rays in vivo. Although radioresistant, the function of effector cells is promptly suppressed by the drug cyclophosphamide. In addition, less differentiated or mature precursors of effector cells are affected by the drug, split-dose irradiation, and the strong adjuvant *C. parvum*. The effect on precursors is inferred from the delayed onset of the suppression of hybrid resistance after preirradiation or *C. parvum*, and from the slow recovery of resistance after all three treatments. Thus, the effector cells appear to belong to a system which renews itself without antigenic stimulation and probably includes the cells reacting to parental and to allogeneic marrow grafts. Within the limits of the experiments described, the effector cells of the two kinds of resistance are indistinguishable. Preliminary observations indicate that these effector cells resemble each other for two additional characteristics: bone marrow origin and thymus independence (1, 25).

The interpretation of hybrid resistance as a host–anti-graft reaction implies that parental cells possess transplantation antigens not shared by F_1 hybrid mice. Even though parental-specific alloantigens have only been detected by transplantation and not by serological techniques, other criteria of transplantation antigens are met (9, 10). They are present in some inbred strains of mice and absent in others, or are highly polymorphic. In most instances the parental antigens are specified by alleles of the major *Hh-1* locus which is part of the *H-2* region (9, 10, 13, 14). Moreover, the antigens in question are tolerogenic under conditions reminiscent of the induction of "high-dose" tolerance to soluble proteins in adult mice. Since this type of tolerance is dependent upon antigen affecting marrow-derived cells (24), the apparent similarity with F_1 hybrid unresponsiveness to parental cells is consistent with the bone marrow origin of effector cells.

The presence of parental transplantation antigens on hemopoietic cells of the mouse strains used in this study is dependent upon *Hh-1* homozygosity (9, 10). This requirement is unique since the other known determinants of *H-2* alloantigens are strictly codominant (16). If parental antigens generate incompati-

bility in both F_1 hybrid and allogeneic mice, their genetic determinants could be regarded as recessive. However, if incompatibility is generated in F_1 hybrids but not in allogeneic mice, then one could assume that the absence of the antigens in F_1 heterozygotes results from suppressive interallelic genetic interactions (9, 10, 13, 14). A second necessary assumption is that only effector cells of *Hh-1* heterozygotes could recognize and react against homozygous marrow cells. To clearly distinguish between the two possibilities, it is essential to establish if resistant inbred mice react against *Hh-1* alloantigens only, *H-2* alloantigens only, or both. The ideal experiment would be the exchange of marrow grafts between mice congenic for *Hh-1* or *H-2* but not for both. Unfortunately, such mice are not available; these genetic determinants are so closely linked that the most discriminant congenic host-donor pairs differ at *Hh-1* and at least the D subregion of *H-2*.

For the reason given above, incompatibility is difficult to analyze in every strain combination and it cannot be excluded that it is generated cumulatively by *Hh-1* and *H-2*. Nevertheless, if *Hh-1* antigens were solely responsible for resistance to marrow grafts in F_1 and inbred-strain mice, one could make the following predictions: (*a*) regulator genes of reactivity, which are determinant-specific (2), should influence both hybrid and allogeneic resistance against given target cells; (*b*) responder mice should always differ from graft donors at the D end of *H-2*; (*c*) marrow cells of *Hh-1–H-2* homozygotes should be strongly rejected by allogeneic recipients but not cells of *Hh-1–H-2* heterozygotes. The first prediction has been verified by finding that the reactivity of several strains of mice decreases in the same order for allogeneic and parental-strain marrow grafts (14, 25). The second prediction was not met since strains of mice unrelated to C57BL are resistant to cells of donors differing at the K end instead of at the D end of *H-2*[2]. The third prediction has been verified by finding that *H-2* heterozygous cells of segregating backcross mice and of F_1 hybrids grow unimpaired in appropriate allogeneic hosts whereas those of homozygous segregants are rejected.[3] While the evidence indicates that *Hh-1* is not responsible for resistance in all host-donor combinations, it does not favor the hypothesis that conventional *H-2* antigens are responsible for marrow graft rejection in allogeneic combinations. It is conceivable that more than one genetic determinant linked to the *H-2* region is inherited like *Hh-1* and that a second *Hh* gene lies in or near the K end of *H-2* (13).

In conclusion, we cannot yet decide whether the genetic determination of *Hh-1* transplantation antigens is recessive or subject to interallelic suppression and, consequently, whether the same or different alloantigens are responsible for the resistance of irradiated mice to parental and allogeneic marrow grafts.

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[2] Cudkowicz, G. Unpublished observations.

[3] Bennett, M. Rejection of marrow allografts: importance of *H-2*-homozygosity of donor cells. In preparation.

SUMMARY

F_1 hybrid mice are capable of rejecting inbred parental strain bone marrow grafts after a single lethal exposure to X-rays. The incompatibility is genetically controlled by the *Hybrid-histocompatibility-1* (*Hh-1*) locus in or near the D end of the *Histocompatibility-2* (*H-2*) region. The onset of parental graft rejection begins 9–12 hr after transplantation and is completed by 24 hr. Maturation of hybrid resistance does not occur until the 22nd day of life. In adults, the resistance to parental marrow grafts can be temporarily abrogated or weakened by administration of cyclophosphamide or dead cultures of *Corynebacterium parvum*, acute supralethal exposures to radiation, or by split-dose irradiation with 6–37-day intervals.

Parental marrow grafts elicit a transplantation reaction in irradiated F_1 mice which is indistinguishable from that elicited in irradiated allogeneic (*H-2*–incompatible) hosts. Because of this immunogenetic similarity, the following question is raised: are the same or different alloantigens responsible for rejection of parental and allogeneic marrow grafts? In the first case, *Hh-1* alleles would be recessive determinants of tissue-specific transplantation antigens, whereas in the second case they would be the determinants of parental- and tissue-specific antigens subject to genetic suppression in *Hh-1* heterozygotes. Although the available evidence is not conclusive in excluding one of the two possibilities, it favors the concept that allograft reactivity to hemopoietic cells is elicited by recessive tissue-specific antigens.

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