PECULIAR IMMUNOBIOLOGY OF BONE MARROW ALLOGRAFTS

I. Graft Rejection by Irradiated Responder Mice*

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Recipients of hemopoietic transplants are usually preexposed to whole body irradiation to deplete the blood-forming system, and so provide the transferred cells with a "graft bed" and stimuli for maximal proliferation and differentiation. It is generally assumed that irradiation in the lethal dose range would also be immunosuppressive and enable allogeneic cells to engraft. This assumption, however, is not correct. In the course of studies on the genetics of hybrid resistance to parental bone marrow grafts, it was noted that allografts did not establish themselves in irradiated hosts of certain mouse strains while succeeding in hosts of other strains (1). The mice in which marrow grafts grew and those in which the same cells failed sometimes belonged to inbred strains sharing the same *H-2* alleles. This suggested that genes other than those specifying major transplantation antigens influenced the outcome of marrow allografts in irradiated mice. Additional evidence is now presented in support of this view. The data also indicate that a peculiar type of incompatibility for hemopoietic allografts is common in irradiated mice, and that it results from destructive host anti-graft reactions. Since the processes of cellular proliferation, antibody formation, and skin graft rejection are impaired after whole body irradiation, bone marrow allografts are presumably rejected by a mechanism previously unknown. This type of allograft reaction is peculiar because it does not require proliferation of lymphoid cells and is tissue specific, thymus independent, and regulated by genetic factors which apparently do not affect the fate of other solid grafts.

Materials and Methods

Mice.—Most inbred and F_1 strains 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], C3H.SW, B10.D2, A.BY), E. S. Russell, Jackson Laboratory

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(WB/Re), J. H. Stimpfling, Columbus Hospital, Great Falls, Mont. (B10.BR, B10.A), and T. S. Hauschka, Roswell Park Memorial Institute, Buffalo, N. Y. (129/Rr, C3H/He, C3H/St, C57BL, A/He, DBA/2Ha). (C3H/HexB10)F_1 mice (abbreviated $C3BF_1$) were obtained from the West Seneca Animal Production Unit of Roswell Park Memorial Institute; C57BL/6, DBA/2J, and (C57BL/6JxDBA/2J)F_1 (abbreviated BDF_1) mice from the Jackson Laboratory; AKR, BALB/c, DBA/2Cum, (BALB/cxDBA/2)F_1, and 101-strain mice from Cumberland View Farms, Clinton, Tenn. 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 700–900 R of total body X-irradiation as described elsewhere (2).

Thymectomy.—The thymus of the adult mouse was aspirated with a suction pipette through an incision in the anterior chest, according to the method described by Miller (3). Control mice were sham operated. The effectiveness of thymectomy was controlled by determining the number of graft-*versus*-host cells in spleens of mice sampled from each group (4).

Immunization.—Mice were given 10^7 viable spleen cells from *H-2*-incompatible donors by intraperitoneal injection. All animals were immunized twice at 7-day intervals and used as graft recipients 4–7 days after the last injection.

Cell Suspensions and Transplantation.—Nucleated bone marrow cells, suspended in Eagle's medium, were counted and injected into a lateral tail vein of irradiated mice as previously described (2, 5).

Assay for Proliferation of Grafted Cells.—5-iodo-2′-deoxyuridine (IUdR)[1] labeled with radioactive ^{125}I was used to assess DNA synthesis in spleens of irradiated mice grafted with bone marrow cells. Details of the method were previously described (2, 5). Briefly, mice were injected with the radioactive DNA precursor 4 or 5 days after transplantation, and the per cent retained in individual spleens was determined by measuring radioactivity with a crystal scintillation counter. The values of IUdR uptake were expressed as arithmetic or geometric means ± standard errors for groups of 4–10 spleens, above the values of control spleens. Controls were irradiated mice not injected with marrow cells; the uptake values of IUdR were not greater than 0.03% in such spleens. The numbers of marrow cells to be grafted were chosen from within the range of linear dose-response relationship, yielding uptake values of IUdR of 0.3–1.0% in syngeneic hosts.

RESULTS

Marrow Graft Failures in Irradiated Mice.—5×10^5–10^6 nucleated marrow cells from B10, DBA/2Ha, or C3H donors were transplanted into syngeneic and allogeneic recipients, 2–4 hr after 700–900 R of X-rays. The allogeneic mice were chosen so that cells of each donor were grafted into recipients sharing the donor's *H-2* allele (*H-2*-compatible strains) and into recipients with different *H-2* alleles (*H-2*–incompatible strains). The latter belonged to sets of two or more strains of mice with identical *H-2* alleles; in this way genetic factors other than *H-2* could vary within each set while the host–donor relationships with respect to *H-2* did not. Whenever possible, the sets of *H-2*–incompatible recipients included mice of a strain congenic (6) with the donor strain for an allelic substitution at the *H-2* locus. These different combinations made it possible to observe the outcome of marrow allografts in recipients differing at several *H*

[1] *Abbreviations used in this paper:* ALS, rabbit anti-mouse thymocyte serum; CY, cyclophosphamide; *Ir*, immune response gene; IUdR, 5-Iodo-2′-deoxyuridine; Thy-X, thymectomy.

loci except *H-2*, at *H-2* and other *H* loci, and at the *H-2* locus only, under the influence of the other genes belonging to different genetic "backgrounds."

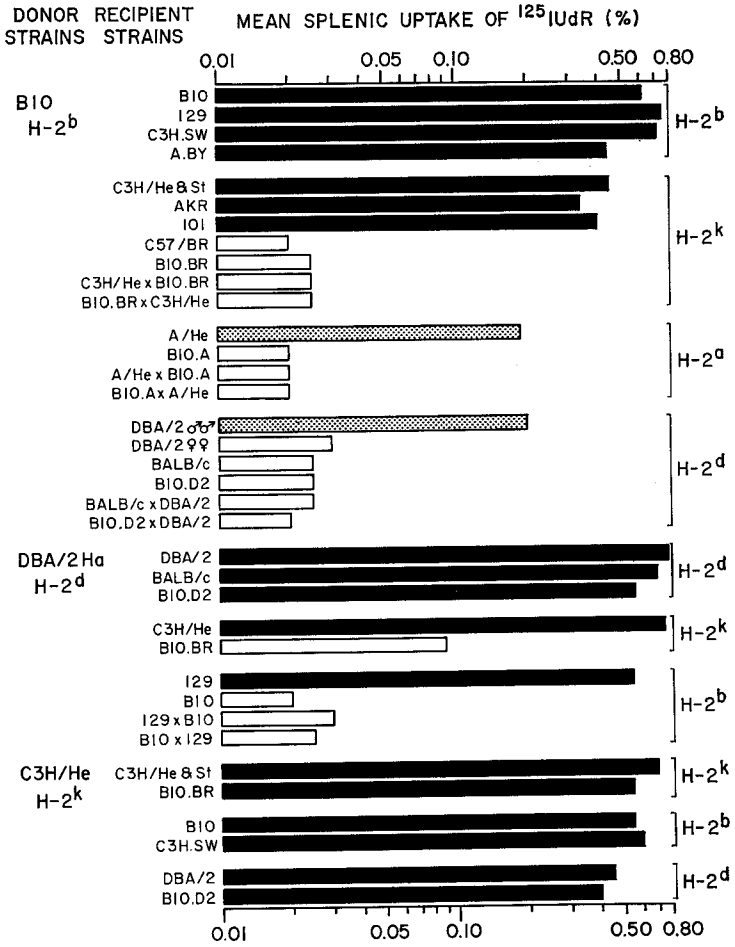

FIG. 1. Proliferation of transplanted marrow cells in syngeneic or allogeneic irradiated recipients. Proliferation was estimated 5 days after transplantation of 0.5–1 × 10^6 cells by measuring splenic retention of the DNA precursor IUdR. Each group of recipients included a minimum of 10 female and 10 male mice grafted in two or more separate experiments. Standard errors of mean values of isotope uptake were 10% or less. Results in DBA/2J, DBA/2Ha, and DBA/2Cum recipients were indistinguishable and, therefore, pooled.

Repopulation of recipient spleens by dividing hemopoietic cells of donor origin was assessed on day 5 after transplantation. The results are presented in Fig. 1.

The mean splenic uptake values of IUdR were similar in syngeneic and in

H-2–compatible allogeneic mice grafted with marrow cells. This indicated that growth of donor cells was not impaired by host–donor differences at loci other than *H-2*. Recipient mice of this type were called "susceptible" to marrow allografts. In *H-2*–incompatible recipients, splenic IUdR uptake values varied according to strain but not to *H-2* type. In each set of *H-2*–incompatible hosts (e.g., AKR, C3H, 101, C57BR, and B10.BR mice, which were all $H\text{-}2^k$ and grafted with B10 cells), IUdR uptake values ranged from ~0.60%, just below, or at the values seen in syngeneic recipients, to 0.01%, the value seen in radiation controls. These *H-2*–incompatible recipients could be classified as resistant, partially resistant, or susceptible, depending on the 5 day growth of transplanted marrow. Except for grafts of B10 marrow cells into DBA/2 recipients, the sexes of donor and recipient mice had no detectable influence on graft proliferation. For this reason the data were not presented separately, although mice of both sexes were used in all experiments. Mice resistant to grafts of one *H-2* type could be partially resistant or even susceptible to grafts of a second type; B10 mice were resistant to DBA/2 but not to C3H grafts, and DBA/2 mice were resistant to B10, but not to C3H grafts. Resistance was not necessarily reciprocal in host–donor pairs; B10 mice were not resistant to B10.BR grafts, although B10.BR mice were resistant to B10 grafts (data not shown in Fig. 1). On the other hand, resistance was reciprocal in mice of the B10 and B10.D2 pair.

Five different matings between susceptible and resistant mice with identical *H-2* alleles were set up to produce *H-2*–homozygous F_1 hybrids. The F_1 mice were given the appropriate test grafts to determine whether resistance or susceptibility would be the dominant inherited trait. Resistance appeared to be phenotypically dominant since the splenic uptake values of IUdR were negligible in all F_1 mice (Fig. 1). Reciprocal hybrids of both sexes, e.g. female and male, (C3H/He x B10.BR)F_1 and (B10.BR x C3H/He)F_1 mice were indistinguishable.

Maturation of Resistance to Marrow Allografts in Infant Mice.—The failure of transplanted marrow cells to proliferate in resistant hosts could have been due to localized graft-*versus*-host reactions (7) or to allogeneic inhibition (8). If so, infant recipients should be at least as resistant as adults, since they are regarded as the most sensitive indicators of graft-*versus*-host reactions and are fully antigenic. 5×10^5 marrow cells of B10 donors were infused into irradiated (600 R) syngeneic and allogeneic recipients that were 15–22 days old. The infant mice were left with their mothers after transplantation. B10.A and B10.D2 recipient mice, which were resistant to B10 cells as adults (Fig. 1), were susceptible at 15, 17, and 20 days of age; they became resistant on day 22 (Table I). 129-strain mice were susceptible as adults and at 22 days of age. The results indicated that resistance was a host function subject to maturation during the first 3 wk of life. This is not compatible with the hypothesis that

marrow graft failure in adult mice resulted from graft-*versus*-host reactivity or from allogeneic inhibition, i.e., the incongruence of donor and host cell surfaces with different *H-2* alloantigens.

Weakening of Resistance to Marrow Allografts with Cyclophosphamide.—Because of the late maturation of resistance in infant mice, it is possible that the failure of marrow grafts in adult mice was due to host anti-graft reactions. If so, the effector mechanisms for rejection of bone marrow and solid tissue grafts must differ, since only the former was not radiosensitive and, presumably, did not require cell proliferation during induction. To investigate this possibility,

TABLE I

*Uptake of IUdR in Spleens of Irradiated Infant Mice 5 Days after Grafting 5×10^5 Bone Marrow Cells of B10 Donors**

Recipient strain	Age	Number and sex of mice		Mean splenic uptake of ^{125}IUdR‡
	(*days*)	(*F*)	(*M*)	(% ± SE)
B10	15	2	4	0.76 ± 0.04
B10.A	16	2	7	0.67 ± 0.03
B10.D2	17	2	1	0.55 ± 0.06
B10	21	2	5	0.45 ± 0.03
129	22	2	4	0.42 ± 0.07
B10.A	20	3	3	0.36 ± 0.08
B10.A	22	6	2	0.08 ± 0.01
B10.D2	22	2	5	0.01 ± 0.004

* Infant mice were exposed to 600 R of X-rays and returned to their mothers regardless of age.

‡ Mean radiation control values (~0.02%) were subtracted from each experimental value.

resistant mice were subjected to immunosuppressive treatment before challenge with the appropriate allografts. Cyclophosphamide was chosen because of its prior use for the induction of hemopoietic chimerism in mice (9–11).

Cyclophosphamide (Cytoxan, Mead Johnson & Co., Evansville, Ind., abbreviated CY) was injected intraperitoneally, 300 mg/kg of body weight, at varying intervals before irradiation (800 R to inbred strain mice and 900 R to F_1 hybrids) and transplantation of 1.3–3.0 $\times$ 10^6 marrow cells. Control mice received injections of saline without CY. The intervals were never shorter than 1 day to avoid cytotoxic effects of the drug on the grafted cells. Repopulation of recipient spleens was assessed 4 days after cell injections; in each experiment one group of mice was treated with CY, irradiated, but not injected with marrow cells to determine the background level of splenic retention of IUdR. Other groups of mice were treated with CY, irradiated, and grafted with syn-

geneic instead of allogeneic cells to determine whether CY influenced grafted cell proliferation in the absence of histoincompatibility. The results are presented in Table II.

B10, DBA/2, and B10.D2 marrow cells grew approximately the same in syngeneic mice injected either with saline or with CY, 1 or 12 days before

TABLE II

*Proliferation of Bone Marrow Cells Grafted into Irradiated Mice Pretreated with Cyclophosphamide**

Donor strain	No. of cells grafted	Recipient strain	Time after CY	Mean splenic uptake of ^{125}IUdR‡ in mice given	
				Saline	CY
	(× 10^6)		(*days*)	(% ± SE)	
B10	2.0	B10	1	0.43 ± 0.07	0.45 ± 0.04
	2.0	B10	12	0.48 ± 0.10	0.42 ± 0.06
	2.3	B10.D2	1	0.03 ± 0.01	0.38 ± 0.06
B10.D2	1.4	B10.D2	1	0.44 ± 0.05	0.27 ± 0.02
DBA/2Ha	1.3	DBA/2Ha	1	1.07 ± 0.13	0.72 ± 0.03
	3.0	B10	1	0.09 ± 0.02	0.58 ± 0.07
	2.0	(B10 x 129)F_1	7	0.05 ± 0.02	0.78 ± 0.07
	2.0	$C3BF_1$	7	0.02	0.78 ± 0.04
	2.0	$C3BF_1$	14	0.02	0.24 ± 0.17
	2.0	$C3BF_1$	21	0.02	0.02§
					0.49 ± 0.52
B10.A	2.3	$C3BF_1$	1	0.02	0.40 ± 0.05
	2.3	$C3BF_1$	7	—	0.21 ± 0.07
	2.3	$C3BF_1$	14	—	0.51 ± 0.10

* Single intraperitoneal injection of 300 mg/kg of CY in saline.

‡ 4 days after irradiation (800–900 R) and transplantation. Radiation control values varied from 0.01 to 0.03% in mice of the strains used, at every interval after CY. Mean radiation control values were subtracted from individual experimental values. 4–10 mice per group.

§ Individual values in this group of six mice were 0.01, 0.01, 0.04, 0.19, 0.42, 1.46.

grafting. CY pretreatment did not increase splenic retention of IUdR in radiation controls at any time interval tested. 1–14 days after CY administration, DBA/2 allografts grew in otherwise resistant B10, (B10 x 129)F_1, and $C3BF_1$ hosts. At 21 days, half of the $C3BF_1$ recipients were still susceptible, while the others had reacquired resistance. B10 allografts grew in otherwise resistant B10.D2 mice 1 day after injection of CY. Likewise, B10.A allografts grew in otherwise resistant $C3BF_1$ mice 1, 7, and 14 days after injection of CY. Thus, CY given to prospective recipients abrogated resistance. However, it did not

alter in a nonspecific way the ability of the host environment to support hemopoietic proliferation, as indicated by IUdR uptake values in spleens of syngeneic CY-treated recipients.

It is noteworthy that another immunosuppressive agent, rabbit anti-mouse thymocyte serum (ALS), prepared as described by Levey and Medawar (12), failed to weaken resistance of C57BL mice to DBA/2Ha marrow grafts. 20 mice were exposed to 800 R of X-rays; one-half were injected intraperitoneally with 0.5 ml of ALS and the remaining with normal rabbit serum 1 hr later. 5×10^5 nucleated marrow cells were grafted on the following day. Both serum treatments had no detectable effect on resistance, since IUdR uptake values in recipient spleens were negligible 5 days after transplantation, as in radiation controls.

Weakening of Resistance to Marrow Allografts with Corynebacterium parvum.— Since the immunosuppressive drug CY weakened the reaction of resistant mice to allogeneic hemopoietic cells, it was of interest to study the effect of a known "immunostimulant" on resistance. *C. parvum* produces in vivo a strong phagocytic response (13), an adjuvant effect on antibody formation and on delayed hypersensitivity (14), and an inhibitory effect on the growth of transplanted tumors (15, 16). Furthermore, it increases the number of hemopoietic progenitor cells in the spleen (17), and presumably enhances in this way resistance of unirradiated F_1 hybrid mice to graft-*versus*-host mortality induced by parental lymphoid cells (18).

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 was injected intravenously into prospective recipients of allogeneic marrow grafts. 7–13 days later the mice were irradiated (800–900 R) and grafted with $0.56–1.12 \times 10^6$ cells. The 7–13-day intervals were chosen because they coincided with the time of maximum phagocytic activity induced by *C. parvum* (13). Repopulation of recipient spleens was assessed 5 days after cell injection. In each experiment one group of mice was treated with *C. parvum*, irradiated, but not injected with bone marrow cells, to determine the background level of splenic retention of IUdR. Other groups of mice were treated with *C. parvum*, irradiated, and grafted with syngeneic instead of allogeneic cells to determine whether *C. parvum* influenced grafted cell proliferation in the absence of histoincompatibility. The results are presented in Table III.

C57BL, B10, and B10.D2 marrow cells grew approximately the same in syngeneic mice given saline or *C. parvum*. 7–13 days after *C. parvum* administration, allografts of cells from these three donor strains grew in otherwise resistant hosts. Hence, resistance was weakened rather than enhanced by *C. parvum*. Transplants of more than 10^6 cells resulted in even greater splenic uptake of IUdR; at intervals of less than 6 days between *C. parvum* and grafting, the weakening effect on resistance was not observed (data not shown).

In some additional experiments, the splenic retention of IUdR was unusually high in radiation controls given *C. parvum*. Presumably, the number of endogenous hemopoietic progenitor cells was increased before irradiation (17) and the fraction of cells surviving irradiation repopulated the spleens. It was, therefore, necessary to obtain additional evidence that IUdR uptake in spleens of allogeneic graft recipients given *C. parvum* was due to proliferation of donor cells. Spleen cells of chimera mice resulting from transplantation of B10 cells into irradiated B10.A hosts given *C. parvum* were retransplanted (one-half

TABLE III

*Proliferation of Bone Marrow Cells Grafted into Irradiated Mice Pretreated with Corynebacterium parvum**

Donor strain	No. of cells grafted	Recipient strain	Time after *C. parvum*	Mean splenic uptake of ^{125}IUdR‡ in mice given			
				Saline		*C. parvum*	
	(× 10^6)		(*days*)	(% ± SE)			
C57BL	0.62	C57BL	7	0.63 ± 0.15	(8)	0.71 ± 0.16	(10)§
		DBA/2Ha ♀♀	7	0.04 ± 0.001	(11)	0.14 ± 0.004	(14)
		B10.D2	7	0.01	(5)	0.31 ± 0.03	(5)
		B10.A	8	0.01	(5)	0.67 ± 0.39	(3)
		B10.A	13			0.12 ± 0.02	(4)
B10	0.56	B10	13	0.68 ± 0.06	(6)	0.60 ± 0.03	(6)
	1.12	B10.A	13	0.02	(8)	0.33 ± 0.09	(9)
B10.D2	1	B10.D2	8	0.32 ± 0.05	(5)	0.38 ± 0.02	(6)
		B10	8	0.02	(6)	0.32 ± 0.04	(17)
		$C3BF_1$	10	0.01	(5)	0.17 ± 0.005	(5)

* Single intravenous injection of 0.5 mg (dry weight) of dead *C. parvum* suspended in saline.

‡ 5 days after irradiation (800–900 R) and transplantation. Radiation control values varied from 0.02 to 0.04 in mice of the strains used, at every interval after *C. parvum*. Mean radiation control values were subtracted from individual experimental values.

§ Number of mice in brackets.

spleen equivalent) on the 7th day into secondary irradiated B10 hosts immunized against antigens of B10.A. In this two-step transfer experiment, B10 marrow cells → B10.A → B10, only donor cells could repopulate the secondary hosts and yield significant IUdR uptake. 8 of 10 spleens of *C. parvum*-treated B10.A recipients contained retransplantable B10 cells, while none of 10 spleens of untreated B10.A recipients did. The weakening of resistance after administration of *C. parvum* was regarded as another evidence in favor of the concept that irradiated mice can elicit destructive host anti-graft reactions. The effect of *C. parvum* also established another difference between the rejection mechanisms for marrow and solid tissue allografts.

Weakening of Resistance to Marrow Allografts by Sublethal Preirradiation.— The lack of immediate sensitivity to radiation of this host anti-marrow allograft reaction could be explained in two ways: (*a*) the effector cells were fixed and nondividing cells, such as neurons, and simply not vulnerable to the antiproliferative activity of the radiation doses used; (*b*) the effector cells were part of a cell-renewal system, but their function, i.e. marrow graft rejection, was not radiosensitive. To distinguish between these two possibilities, mice resistant to allogeneic cells of given strains, and syngeneic control mice, were

TABLE IV

Proliferation of 10^6 Transplanted Bone Marrow Cells in Mice Exposed to Split-Dose Irradiation

Donor strain	Recipient strain	Exposures to X-rays	Interval between exposures*	Mean splenic uptake of ^{125}IUdR‡
			(*days*)	
C57BL	B10	500 + 700	7	0.46 ± 0.06 (5)§
	C57BL	700	—	0.43 ± 0.03 (3)
	B10.D2	500 + 700	7	0.24 ± 0.04 (5)
	B10.D2	700	—	0.01 (5)
C57BL	C57BL	300 + 700	7	0.52 ± 0.07 (5)
	C57BL	400 + 700	7	0.60 ± 0.03 (8)
	C57BL	700	—	0.60 ± 0.15 (5)
	B10.BR	300 + 700	7	0.16 ± 0.05 (6)
	B10.BR	400 + 700	7	0.14 ± 0.04 (7)
	B10.BR	700	—	0.02 (5)
DBA/2Ha	C57BL/6	400 + 750	14	0.94 ± 0.25 (22)
	C57BL/6	750	—	0.01 (10)

* Second exposure to X-rays preceded marrow grafting by a few hours.

‡ 5 days after transplantation. Radiation control values varied from 0.01 to 0.03% in mice of the strains used after split-dose irradiation. Mean radiation control values were subtracted from individual experimental values.

§ Number of mice in brackets.

exposed to sublethal irradiation (300–500 R) 7 or 14 days before a second exposure (700–900 R) and challenge with marrow grafts of the appropriate strains. The results are reported in Table IV.

10^6 C57BL marrow cells grew approximately the same on transplantation into preirradiated or untreated syngeneic recipients. Preirradiation of allogeneic mice weakened resistance. Proliferation of C57BL cells occurred in otherwise strongly resistant B10.D2 and B10.BR mice, and proliferation of DBA/2 cells occurred in preirradiated C57BL/6 mice. Additional experiments, not reported in Table IV, indicated that the weakening of resistance did not occur until 6 days after preirradiation.

Transfer of Resistance and Susceptibility With Allogeneic Bone Marrow Cells.— The decreased strength of resistance several days after administration of *C. parvum* or after exposure to sublethal doses of X-rays, and the late maturation of resistance in infant mice suggested that a population of host cells undergoing renewal is endowed with the ability to reject bone marrow allografts. To determine whether these effector cells belonged to the hemopoietic system, bone marrow cells were exchanged between mice sharing the same *H-2* allele but differing for resistance to a third party graft.

129-strain mice, susceptible to DBA/2 marrow cells, were exposed to 750 or 820 R of X-rays in two separate experiments and were infused with 10^7 marrow cells from either syngeneic or C57BL donors. Mice of both strains, 129 and C57BL, are $H\text{-}2^b$ homozygotes. Cell transfers were also done in the opposite direction in two other experiments, i.e., 129 or B10 marrow cells were injected into irradiated B10 recipients. 60–63 days later, surviving mice were presumably blood chimeras. At this time the chimeras were exposed to radiation for the second time (700–800 R) and given a test graft of 10^6 DBA/2Ha ($H\text{-}2^d$) marrow cells. Splenic repopulation was assessed 5 days later. A fifth experiment with C57BL/6 mice as recipients was done in a similar way; the mice were initially exposed to 400 R of X-rays, injected with 4×10^6 129-strain or syngeneic marrow cells, and challenged with DBA/2Ha cells 13 days later. The results are shown in Table V

60–63 days after irradiation and repopulation with syngeneic bone marrow cells, 129-strain and B10 mice were as susceptible and resistant, respectively, to DBA/2 grafts, as untreated control mice. The B10 mice presumably lost resistance shortly after irradiation and transplantation (Table IV), but were reconstituted by the syngeneic graft. The phenotype of 129-strain and B10 chimera mice repopulated with allogeneic cells changed. 129-strain chimeras became resistant, whereas B10 chimeras became susceptible to DBA/2 grafts. The allogeneic chimeras assumed the phenotype of the donor's blood-forming tissue with respect to resistance or susceptibility. The same results were obtained when C57BL/6 mice were repopulated with fewer 129-strain marrow cells and challenged with DBA/2 grafts at a much shorter interval. Bone marrow-derived cells conferred resistance or susceptibility in these experiments irrespective of the B10 or 129-strain environments, presumably by generating effector cells for DBA/2 graft rejection.

In contrast to bone marrow cells, serum did not passively transfer resistance. 129-strain susceptible mice and $C3BF_1$ resistant mice (Tables II and V) were irradiated (800 and 900 R, respectively) and grafted with 1.28×10^6 marrow cells from DBA/2Ha donors. 18 hr later, at the time of graft rejection (see Fig. 2), $C3BF_1$ mice were bled and 0.5 ml of serum was injected into the peritoneal cavity of the 129-strain recipients of DBA/2Ha cells. Untreated $C3BF_1$ and irradiated $C3BF_1$ mice not injected with DBA/2Ha cells were also

used as serum donors. None of the three sera influenced to any extent the growth of DBA/2Ha cells in 129-strain mice. Sera from resistant and susceptible mice of several strains were not cytotoxic for marrow cells of appropriate genotype in the presence of guinea pig or rabbit complement in vitro and in vivo.

TABLE V

*Cellular Transfer of Resistance and Susceptibility to Bone Marrow Allografts; Proliferation of DBA/2 Test Grafts in Reciprocal C57BL to 129-Strain Chimeras**

Original donor strain	Recipient strain	Induction of Chimerism		Duration of chimerism	Mean splenic uptake of ^{125}IUdR‡
		X-Ray exposure	No. of marrow cells grafted		
		(*R*)	(× 10^6)	(*days*)	(% ± SE)
B10	129	750	10	63	0.08 ± 0.08 (5)§
129		750	10	63	0.57 ± 0.09 (9)
None		—	—	—	0.35 ± 0.10 (7)
C57BL/6	129	820	10	60	0.06 ± 0.10 (5)
129		820	10	60	0.67 ± 0.03 (4)
None		—	—	—	0.42 ± 0.14 (5)
129	B10	750	10	63	0.22 ± 0.07 (6)
B10		750	10	63	0.01 (10)
None		—	—	—	0.01 (5)
129	B10	770	10	60	0.48 (2)
B10		770	10	60	0.03 (8)
None		—	—	—	0.04 (5)
129	C57BL/6	400	4	13	0.71 ± 0.25 (10)
C57BL/6		400	4	13	0.05 ± 0.06 (6)
None		—	—	—	0.01 (2)

* Test grafts of 10^6 DBA/2 bone marrow cells were injected into chimeras and untreated control mice a few hours after exposure to 700–800 R of X-rays. For the chimeras this was the second exposure to radiation.

‡ 5 days after transplantation of DBA/2 cells. Mean radiation control values were subtracted from individual experimental values.

§ Numbers of mice in brackets.

The Effect of Thymectomy on Differentiation of Effector Cells.—Since rejection of marrow allografts was due to cells of the hemopoietic system, it became of interest to determine whether differentiation of effector cells was thymus dependent. C57BL/6 and $C3BF_1$ mice were thymectomized or sham operated at 9 wk of age. 2 wk later, the animals were exposed to 750–850 R of X-rays and reconstituted with 10^7 syngeneic marrow cells. 7 or 9 wk after reconstitu-

tion, the mice were reexposed to X-rays (750 R for C57BL/6 and 900 R for $C3BF_1$) and challenged with DBA/2, WB, or BDF_1 marrow allografts. Splenic repopulation was assessed on the 4th day. Thymectomized and sham-operated $C3BF_1$ mice were also given syngeneic test grafts to control that surgical procedures did not interfere with splenic repopulation (Table VI). Resistance was not weakened in any of 18 thymectomized and 22 sham-operated mice, not

TABLE VI

Proliferation of Bone Marrow Test Grafts in Mice which were Thymectomized, Irradiated, and Reconstituted with Syngeneic Marrow

Syngeneic chimera*			Test donor		Mean splenic uptake of ^{125}IUdR‡
Strain	Pretreatment	Duration	Strain	No. of cells grafted	
		(*days*)		(× 10^6)	(% ± SE)
C57BL/6	Sham Thy-X	64	DBA/2Ha	1	0.03 (5)§
	Thy-X	64		1	0.01 (4)
$C3BF_1$	Sham Thy-X	49	DBA/2Ha	1.4	0.04 ± 0.01 (5)
	Thy-X	49		1.4	0.06 ± 0.01 (4)
$C3BF_1$	Sham Thy-X	49	WB	1.5	0.01 (4)
	Thy-X	49		1.5	0.01 (4)
$C3BF_1$	Sham Thy-X	49	BDF_1	1.5	0.18 ± 0.03 (8)
	Thy-X	49		1.5	0.12 ± 0.07 (6)
$C3BF_1$	Sham Thy-X	49	$C3BF_1$	1.4	0.54 ± 0.08 (4)
	Thy-X	49		1.4	0.76 ± 0.08 (5)

* Mice were thymectomized (Thy-X) at the age of 9 wk; they were irradiated (750–850 R) and reconstituted with 10^7 syngeneic marrow cells 2 wk later.

‡ 4 days after transplantation of allogeneic or syngeneic test grafts. Mean radiation control values were subtracted from individual experimental values. Viability of donor cells was verified by transplantation into syngeneic untreated recipients (data not reported).

§ Number of mice in brackets.

even in the $BDF_1 \rightarrow C3BF_1$ combination in which resistance was partial. Hence, neither inductive nor suppressive thymic influences were required in adult mice for differentiation of the marrow-derived effector cells.

Time of Rejection of B10 Marrow Allografts by Irradiated Resistant Hosts.— Viable marrow cells grafted into irradiated allogeneic resistant mice did not generate proliferating progeny within 4–5 days. Graft failures could have resulted either from killing of the hemopoietic progenitor cells of donor origin, i.e. rejection, or from inhibition of donor cell proliferation and differentiation without killing. To test for survival of donor cells in spleens of irradiated

resistant mice, the spleens were sampled 3–120 hr after transplantation and retransplanted into irradiated secondary recipients syngeneic with the original marrow donor. The secondary recipients were actively immunized against antigens of the primary recipients which were resistant to the donor cells. 2.5×10^6 B10 marrow cells were transplanted into each irradiated (800 R)

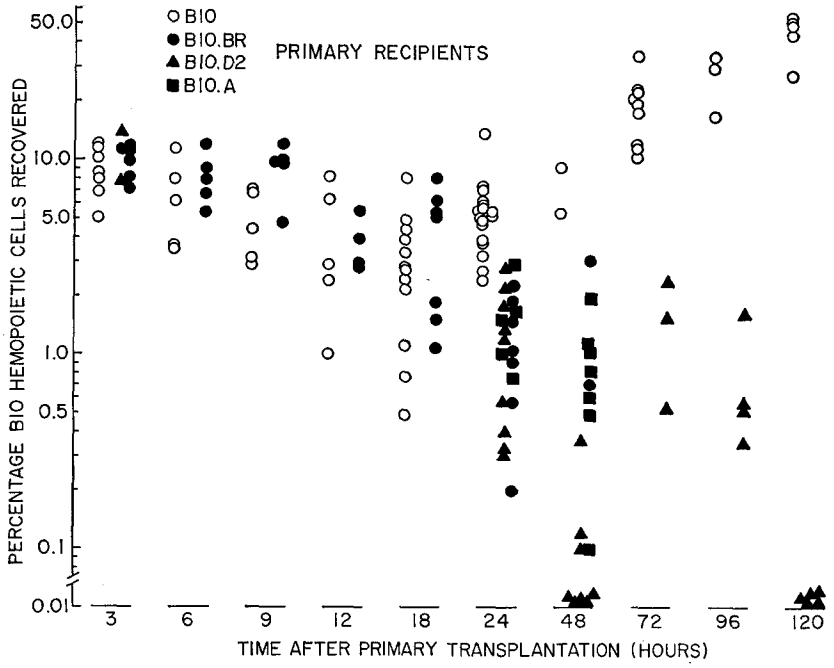

FIG. 2. Recovery of retransplantable B10 hemopoietic cells from the spleens of recipient mice at various times after transplantation of 2.5×10^6 marrow cells. Primary recipients were mice of strains B10 (syngeneic with donor cells), B10.D2, B10.BR, and B10.A (allogeneic with and resistant to donor cells). Points represent the percentages of cells recovered from the whole spleens of individual primary recipients, as estimated by retransplantation into secondary B10 recipients. The 100% value was estimated from primary B10 recipients given 2.5×10^5 marrow cells.

mouse of the following strains: B10(syngeneic), B10.D2, B10.BR, and B10.A (allogeneic with and resistant to B10). Female and male mice were used, but sexes of donors and recipients were always matched. At the intervals indicated in Fig. 2, primary recipient mice were killed and the spleen cells dispersed to determine the content of retransplantable donor-type hemopoietic cells. All secondary B10 hosts were exposed to 800 R of X-rays and each mouse was grafted with the cells of one whole primary recipient spleen; these B10 mice were preimmunized against B10.D2, B10.BR, or B10.A alloantigens (or

injected with B10 spleen cells) before irradiation depending upon the strain of the primary recipients. Proliferation of donor-type hemopoietic cells was assessed in secondary recipients 5 days after retransplantation. For each experiment a group of irradiated B10 mice was injected with 2.5×10^5 marrow cells of the original donor cell preparation instead of 2.5×10^6, to measure proliferation of progenitor cells in terms of 5 day splenic uptake of IUdR without retransplantation. The mean value so obtained was multiplied by 10 to estimate the value of splenic uptake of IUdR which would have been obtained in secondary hosts if 100% of progenitor cells injected into primary recipients could have been recovered. The percentages of cells actually recovered from each of the 70 syngeneic and 79 resistant primary recipient mice are shown in Fig. 2.

During the first 12 hr after transplantation of B10 marrow cells into syngeneic mice, only a small percentage of the injected progenitor cells was recovered from the host spleens. With increasing time, recovery tended to decrease with a nadir at 18 hr. Afterwards, recovery increased at an exponential rate, owing to replication and differentiation of transplanted stem cells (17). In allogeneic hosts resistant to B10 marrow grafts, recovery of hemopoietic cells was comparable to that in syngeneic hosts for the first 18 hr. At 24 hr a small fraction of transplanted cells, approximately 1%, was still reproductively viable. At this time, however, recovery was less from the spleens of most resistant hosts than from B10 mice. At later intervals the discrepancy between B10 and resistant mice became greater owing to expansion of hemopoietic cell numbers in the former and loss of cells in the latter. B10 stem cells did not proliferate or differentiate in allogeneic resistant mice, since neither new progenitor cells nor differentiating descendents were produced. Stem cells also did not survive beyond 96 hr. In view of the possible survival and function of B10 stem cells in infant allogeneic mice or in adults whose resistance had been weakened (Tables II–IV), it is concluded that the B10 cells were actually rejected beginning 18–24 hr after transplantation.

DISCUSSION

The failure of transplanted bone marrow cells to engraft in irradiated but otherwise untreated allogeneic mice of certain strains is due to an active host anti-graft reaction directed specifically against hemopoietic target cells bearing *H-2* alloantigens. This conclusion is primarily based on the following observations: (*a*) graft failure can be prevented by several kinds of host pretreatments which are either anti-proliferative and cytocidal (cyclophosphamide, split-dose irradiation), or intensely stimulating to the reticular system (*C. parvum*); (*b*) marrow graft failure does not occur in infant mice during the 2nd and 3rd week of life; (*c*) mice of resistant and susceptible strains are equally incompatible to skin and other solid tissue allografts, irrespective of whether sus-

ceptibility to bone marrow was a genetic trait or induced by *C. parvum* (6, 15, 16); (*d*) for marrow graft failure to occur the donor and recipient strains must differ at the major histocompatibility locus *H-2*, but not necessarily at other minor *H* loci.

A limited survey of 14 inbred mouse strains for resistance to marrow allografts after whole body irradiation clearly indicated that the outcome of the grafts was extremely variable and not predictable by the *H-2* alleles of donor and recipients. *H-2* incompatibility was a necessary but not sufficient prerequisite for resistance to marrow grafts in several host–donor combinations. For example, mice of three $H\text{-}2^k$ strains, C3H, AKR, and 101, were susceptible to B10 grafts, while mice of two others, C57BR and B10.BR, were not. It is to be noted that the B10.BR strain was congenic with B10, thus differing from the donor strain only at the *H-2* locus. However, host–donor differences at several other minor *H* loci (with or without *H-2* differences) did not result in marrow graft failure in host mice of the congenic pair C3H ($H\text{-}2^k$) and C3H.SW ($H\text{-}2^b$) given B10 cells. The variable outcome of B10 grafts in series of allogeneic recipient strains of the same *H-2* type indicated that other genetic factors controlled recognition of, or reactivity to, *H-2* alloantigens of hemopoietic cells, or both. The same could be said for B10 grafts in mice of $H\text{-}2^a$ and $H\text{-}2^d$ strains, and for DBA/2 grafts in mice of $H\text{-}2^k$ and $H\text{-}2^b$ strains (Fig. 1). The interstrain variation and the dominant inheritance of resistance by F_1 mice in five different crosses strongly supported this view. Since mice of given strains were not universal acceptors or rejectors of *H-2*–incompatible grafts, the genetic factors involved were determinant specific and presumably controlled recognition rather than reactivity. Resistant or susceptible phenotypes were expressed by marrow-derived host cells and not by other cell types or by the environment. These features of marrow graft rejection resemble those of specific antibody responses to natural and synthetic antigens regulated by *immune response* (*Ir*) genes (19) and those of resistance to leukemogenic viruses controlled by the *Fv-1* gene (20). Accordingly, mice resistant to marrow allografts could be regarded as genetic "responders," susceptible mice as "nonresponders," and partially resistant mice as "poor responders."

It has been emphasized that *Ir* genes are closely linked with, or an integral part of, *H* genes (19, 21–23), and that the broader category of genes regulating defense reactions may also be functionally related to *H* loci (24). Recognition of antigens, alloantigens, and viruses may be influenced by patterns on cell surfaces specified by *H* genes. If this were the case for certain chromosomal regions regulating marrow graft rejection, one would expect inbred mouse strains of common ancestry and with relatively small degree of diversity for *H* alleles to exhibit limited diversity also for resistance or susceptibility to given *H-2*–incompatible grafts, and vice versa. According to the survey presented in Fig. 1, most strains resistant to B10 and DBA/2 grafts belong to the

ancestry group with minimal diversity for minor *H* loci, identified as group No. 2 by Graff and Snell (25). Conversely, the susceptible strains belong either to ancestry group No. 1 of greater diversity or to ancestry group No. 3. Irrespective of whether certain minor *H* genes themselves or *H*-linked genes regulated marrow graft rejection, these studies showed that donor and host strains do not have to differ at these loci. It is tempting to speculate that congruence of critical cell surface areas, such as those specified by minor *H* genes, would facilitate recognition of incongruent areas containing *H-2* alloantigens. From the survey made it appeared that host resistance was stronger for marrow allografts of congenic donors differing only at the *H-2* region than for marrow grafts of unrelated strains. The number of determinant-specific genes regulating marrow graft rejection in the susceptible-resistant pairs C3H/He-B10.BR and B10-129 (grafted with B10 and DBA/2 cells, respectively) was tentatively estimated to be a minimum of two for each pair (26).

In addition to the peculiar genetics, physiologic parameters of marrow allograft rejection are also unlike those of other transplantation reactions. The persistence of reactivity after 700–900 R of whole body X-irradiation and the rapid onset of rejection 24 hr after transplantation are indirect evidence that host cell proliferation is not required. Relative radio-resistance is not so unusual for cell-mediated immune responses since delayed-type hypersensitivity reactions (27, 28), normal lymphocyte transfer reactions (29), and production of homocytotropic antibody (30) develop after radiation exposures suppressive for production of other humoral antibodies. If cellular proliferation does not take place between marrow grafting and rejection, effector cells endowed with specificity could be induced directly by antigenic cells as a final step in maturation. However, effector cells could be generated independently of antigen in the course of the normal cellular differentiation, maturation, and proliferation of the immune system (31). The "spontaneous" maturation of resistance in infant mice of responder strains, and the weakening of resistance by preirradiation of prospective recipients or by their pretreatment with *C. parvum* indicated that at least all but the final step in differentiation are antigen independent. "Spontaneous" occurrence of delayed-type hypersensitivity to alloantigens has been described in guinea pigs by Battisto (32).

The transfer with bone marrow cells of resistance and susceptibility to irradiated hosts of opposite phenotype is taken as evidence that effector cells for marrow graft rejection are derived from bone marrow, as are effector cells in delayed hypersensitivity (33, 34) and in skin graft rejection (35). Differentiation of effector cells is thymus independent both for marrow graft rejection and for delayed-type hypersensitivity reactions (33). However, the former, unlike the latter, do not require thymus-derived cells even for initiation of marrow graft rejection. The effector cells presumably are not phagocytes since *C. parvum* weakens resistance at the time of enhanced phagocytic activity. It

is possible that the unexpected effect of *C. parvum* on resistance was due to exhaustion or diversion of common precursors of phagocytes, lymphoid cells, and effector cells. The bone marrow origin of cells responsible for graft rejection is consistent with the results of tests in which allogeneic fibroblastic cells were killed in vitro by irradiated lymphoid cells of thymectomized irradiated mice reconstituted with marrow (36).

Marrow allograft failures in irradiated hosts could have resulted from properties inherent to the transplanted cells rather than to host reactivity. Specifically, lymphoid cells of transplanted marrow could have engaged in graft-*versus*-host reactions and destroyed bystander hemopoietic stem cells (7). This was unlikely because mouse bone marrow grafts do not cause acute graft-*versus*-host reactions. However, the optimal growth of marrow allografts in infant mice, which are exquisitely sensitive to graft-*versus*-host disease, and the similar fate of transplanted marrow and graft-*versus*-host cells in responder and nonresponder mice (4), make this hypothesis untenable. The so-called phenomenon of allogeneic inhibition (8), by which cells die after exposure to alloantigens, failed to occur in nonresponder mice and in young or appropriately pretreated adult responder mice. It cannot explain, therefore, the resistance of irradiated responder mice to bone marrow allografts.

SUMMARY

Mice are capable of rejecting *H-2*–incompatible bone marrow grafts after a single lethal exposure to X-rays. The onset of rejection begins 18–24 hr after transplantation and is completed by 96 hr. Maturation of this type of allograft reactivity does not occur until the 22nd day of life. In adult mice, the resistance to marrow allografts can be weakened by administration of cyclophosphamide or dead cultures of *Corynebacterium parvum*, but not heterologous anti-thymocyte serum. Sublethal exposures to X-rays 7 or 14 days before transplantation also weaken resistance. There is considerable interstrain variation in the ability of mice to resist allografts, even when *H-2* differences between hosts and donor are kept identical. Although *H-2* incompatibility is a necessary prerequisite for resistance, additional genetic factors influence the outcome of marrow allografts, presumably by controlling recognition. The regulator genes are determinant specific and the alleles for resistance or responder status appear to be dominant. The responder phenotype is expressed by hemopoietic cells and not by the environment. Accordingly, resistance is conferred to otherwise susceptible mice upon transfer of bone marrow cells but not of serum. The production and differentiation of effector cells for marrow graft rejection are thymus independent.

In conclusion, bone marrow allografts elicit a particular transplantation reaction, previously unknown, in irradiated mice. Peculiar features of this reaction are the lack of proliferation of host lymphoid cells, tissue specificity,

thymus independence, and regulation by genetic factors which apparently do not affect the fate of other grafts.

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