ACUTE REJECTION OF MURINE BONE MARROW ALLOGRAFTS BY NATURAL KILLER CELLS AND T CELLS

Differences in Kinetics and Target Antigens Recognized

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The nature of the effector mechanisms and target antigens involved in the rejection of bone marrow allografts has been controversial. This has mainly been due to differences in the experimental model systems and the times used for examining rejection. Acute rejection of bone marrow allografts is believed to occur through the recognition of Hemopoietic histocompatibility (Hh)[1] determinants present in incompatible stem cells (1). H-2-homozygous bone marrow cells (BMC) are rejected, whereas stem cells from H-2-heterozygous donors are not (2). It was postulated that Hh determinants are recessively inherited and therefore not expressed on H-2 heterozygous cells. The rejection of certain parental strain BMC by their F_1 hybrids (hybrid resistance) is consistent with the idea that Hh antigens are not inherited codominantly (3). The most widely investigated Hh antigens involved in marrow graft rejection, Hh-1 antigens, are coded for by genes within the H-2 complex. Recent mapping studies place the Hh-1 locus between H-2S and H-2D (4), suggesting that Hh-1 determinants are probably not class I antigens. However, some studies have demonstrated that H-2-heterozygous marrow grafts, which are expected to be Hh-1^-, can be resisted by lethally irradiated mice under certain experimental conditions (5, 6). This would suggest that determinants other than Hh-1 (i.e. class I MHC antigens) may play a role in the rejection of marrow grafts.

Natural killer (NK) cells mediate specific marrow allograft rejection in the absence of functional T or B cells (7). NK cells are responsible for the phenomenon of hybrid resistance to parental marrow grafts (3, 8). Although NK cells have been implicated as anti-Hh effector cells, it is generally believed that NK cells are a functionally defined population of nonspecific killer cells that would be unlikely to specifically recognize incompatible stem cells. Therefore, another theory to explain the mechanism of NK cell–mediated marrow allograft rejection has been proposed. When an NK cell encounters an allogeneic stem cell, failure to recognize self MHC determinants on the stem cell results in the destruction of that cell (9). Allogeneic marrow grafts would then be rejected due to a lack

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[1] *Abbreviations used in this paper:* BMC, bone marrow cells; Hh, hemopoietic histocompatibility; SC, spleen cells; SCID, severe combined immunodeficiency.

of self MHC determinants on the cells rather than due to the presence of allogeneic determinants.

While NK cells have been demonstrated to be effector cells in murine marrow graft rejection, proof that NK cells can mediate marrow allograft rejection in other species is lacking. In addition, evidence that radioresistant T cells can respond to alloantigens is mounting (6, 10, 11). Recent studies have demonstrated that lymphocytes can be sensitized after lethal irradiation and cause the rejection of skin allografts in rats (12). Studies with primates and humans have also implicated a role for T cells in the rejection of bone marrow transplants (13, 14). In experiments reported here we have performed a systematic analysis of the potential effector mechanisms responsible for the acute rejection of marrow allografts in lethally irradiated mice. We observed that both NK cells and T cells can be the effectors mediating the acute rejection of bone marrow allografts after lethal irradiation. However, they differ with respect to the target antigens recognized and the kinetics of the rejection response.

Materials and Methods

Mice. C57BL/6J (B6), DBA/2J, BALB/cJ, (C3H × C57BL/6)F_1 (C3B6F1), (C3H × DBA/2)F_1 (C3D2F1), C.B-17 *scid/scid* (SCID), and C.B-17 +/+ mice were bred in our own colony at the University of Texas Health Science Center. C.B-17 *scid* and C.B-17 +/+ mice were kept in specific pathogen–free conditions until use. BALB/c *nu/nu* (nude) and BALB/c +/+ (euthymic) mice were purchased from The Jackson Laboratory (Bar Harbor, ME). Mice were 8–16 wk old when used. Before irradiation, mice were given acid water (pH 2.5) and neomycin sulfate (Biosol liquid; Upjohn Co., Kalamazoo, MI), 1 ml in 500 ml tap water.

Irradiation. Recipient mice were exposed to 450–850 cGy of ^{137}Cs gamma radiation at a dose rate of 85.5 cGy/min. The exposure doses were: SCID mice, 450 cGy; BALB/c *nu/nu*, BALB/c +/+, and C.B-17 +/+ mice, 700 cGy; DBA/2 and B6 mice, 800 cGy; and C3B6F1 and C3D2F1 mice, 850 cGy.

Pretreatment of Mice. The anti-NK1.1 mAb (PK136) reagent was the generous gift of Dr. G. Koo (Merck, Sharpe, and Dohme, Inc., Rahway, NJ). PK136 in ascites form (200 μg) was injected intraperitoneally 2 d before BMC transfer (15). The anti-Lyt-2 mAb (HO-2.2) was a gift from Delanie Cassell (University of Texas Health Science Center). HO-2.2, in ascites form, was injected (100 μl) intraperitoneally at daily intervals starting 3 d before BMC transfer and ending 1 d after BMC transfer. In one experiment, mice were injected with 120 μg poly(I:C) intraperitoneally on the day of irradiation and marrow cell transfer.

Immunization Protocol. Spleen cell (SC) and BMC were harvested from donor mice and suspensions of cells (3:1 SC/BMC) were exposed to 900 cGy. 10^7 cells were injected together with the original BMC innoculum intravenously into the lethally irradiated recipients.

Assay for Marrow Cell Proliferation. The assay for the proliferation of grafted hemopoietic cells in lethally irradiated mice has been described previously (16). Briefly, recipient mice are lethally irradiated and infused with 1–4 × 10^6 BMC into a lateral tail vein. The proliferation of donor cells in the spleens of the recipient mice was measured 4–8 d later by measuring the splenic incorporation of 5-^{125}I-iodo-2′-deoxyuridine (^{125}IUdR), a specific DNA precursor and thymidine analogue. Each mouse was injected with 10^{-7} M fluorodeoxyuridine intraperitoneally to inhibit endogenous thymidylate synthetase. 1 h later each mouse was injected intraperitoneally with 0.5 μCi ^{125}IUdR (Amersham Corp., Arlington Heights, IL). 4 h after injection of isotope, the animals were sacrificed and their spleens were removed. The spleens were soaked in 70% ethanol for 2 d to elute non-DNA ^{125}I, and the ^{125}I radioactivity retained was measured in a well-type gamma counter. The data are presented as the geometric means (with a range of 95% confidence

TABLE I

Acute Rejection of H-2-Heterozygous and -Homozygous Marrow Allografts by Lethally Irradiated SCID, Nude, and +/+ Recipients

Exp.	Marrow donor		Cells grafted	Recipient		Day of assay	Splenic ^{125}IUdR uptake	
	Strain	H-2		Strain	H-2		Geometric mean	95% Confidence limits
			$\times 10^6$					
1	B6	b/b	2.5	B6	b/b	4	0.15	0.10–0.23
	B6	b/b	2.5	+/+	d/d	4	0.03	0.01–0.06*
	B6	b/b	2.5	Nude	d/d	4	0.03	0.01–0.08*
2	B6	b/b	3.2	B6	b/b	5	0.43	0.28–0.66
	B6	b/b	3.2	+/+	d/d	5	0.30	0.15–0.60
	B6	b/b	7.5	+/+	d/d	5	0.83	0.39–1.75
	B6	b/b	7.5	SCID	d/d	5	0.11	0.02–0.49*
	C3B6F1	k/b	2.5	C3B6F1	k/b	5	1.01	0.70–1.41
	C3B6F1	k/b	2.5	+/+	d/d	5	1.15	1.00–1.31
	C3B6F1	k/b	2.5	SCID	d/d	5	0.81	0.74–0.90
	C3B6F1	k/b	2.5	Nude	d/d	5	0.76	0.37–1.53

SCID = C.B-17 *scid/scid*; +/+ = C.B17 +/+; nude = BALB/c *nu/nu*. All recipients in Exp. 1 were injected with poly(I:C) at the time of cell transfer.

* Geometric mean value significantly ($p < 0.05$) lower than syngeneic controls.

limits) of percentage of injected ^{125}IUdR incorporated into the spleens of groups of five to eight mice. Irradiated recipients syngeneic with the BMC donor were used to assess the growth potential of the grafted BMC (syngeneic controls). Lethally irradiated mice not injected with viable BMC served as radiation controls. Both parametric and nonparametric statistical analyses were performed to determine if the geometric values of various groups differed significantly ($p < 0.05$) (17). The inoculum sizes used for a given time were chosen to ensure that the isotope uptake values were on the exponential part of the curve relating numbers of BMC grafted to percent splenic ^{125}IUdR uptake (16).

Results

Differential Rejection of H-2-Homozygous and -Heterozygous Allogeneic BMC Grafts. Both C.B-17 *scid/scid* (SCID) and BALB/c *nu/nu* (nude) mice are capable of rejecting H-2-homozygous allogeneic BMC grafts (7, 18). SCID mice lack both functional B cells and T cells due to a defect in immune receptor gene rearrangement (19). SCID mice completely lack any T cell function (e.g. will not reject skin allografts) yet display normal NK activity (20). This knowledge allowed us to determine whether or not Hh-1 antigens are recognized during rejection, or if the self–nonself hypothesis is correct; e.g., that NK cells lyse or inactivate those potential target cells that do not share H-2 determinants with the NK cells. We therefore challenged lethally irradiated C.B-17 +/+ (+/+), BALB/c *nu/nu* (nude), and C.B-17 *scid/scid* (SCID) mice (all H-2^d), with H-2-homozygous B6 (H-2^b, Hh-1^b) or H-2-heterozygous C3B6F1 (H-2^k/H-2^b, Hh-1^{null}) BMC grafts. Splenic ^{125}IUdR uptake was measured 4 or 5 d after BMC transfer. In Exp. *1*, Table I, prospective recipient mice were injected with poly(I:C) to boost NK cell function and marrow allograft reactivity (7). Both nude and +/+ hosts resisted

TABLE II

Kinetics of Rejection of H-2-Heterozygous Allogeneic BMC by Immunized Hosts

Exp.	Marrow donor		Cells grafted*	Recipient		Day of assay	Splenic ^{125}IUdR uptake	
	Strain	H-2		Strain	H-2		Geometric mean	95% Confidence limits
			$\times 10^6$					
1a	C3B6F1	k/b	2.5	C3B6F1	k/b	4	1.57	0.93–2.63
	C3B6F1	k/b	2.5	SCID	d/d	4	1.10	0.78–1.55
	C3B6F1	k/b	2.5	+/+	d/d	4	1.03	0.18–5.69
1b	C3B6F1	k/b	1	C3B6F1	k/b	7	2.80	1.31–5.96
	C3B6F1	k/b	1	+/+	d/d	7	0.66	0.28–1.53‡
	C3B6F1	k/b	1	+/+§	d/d	7	0.09	0.04–0.20‡
2a	C3B6F1	k/b	2.5	C3B6F1	k/b	4	0.39	0.14–1.13
	C3B6F1	k/b	2.5	+/+	d/d	4	0.36	0.32–0.41
	C3B6F1	k/b	2.5	+/+§	d/d	4	0.56	0.39–0.81
2b	C3B6F1	k/b	1	C3B6F1	k/b	7	1.05	0.48–2.28
	C3B6F1	k/b	1	+/+	d/d	7	1.85	0.66–5.55
	C3B6F1	k/b	1	+/+§	d/d	7	0.07	0.01–0.17‡
3a	C3D2F1	k/d	2.5	C3D2F1	k/d	4	0.52	0.22–1.22
	C3D2F1	k/d	2.5	B6	b/b	4	0.54	0.37–0.79
3b	C3D2F1	k/d	1	C3D2F1	k/d	7	1.23	0.42–3.56
	C3D2F1	k/d	1	B6	b/b	7	0.40	0.30–0.79‡
	C3D2F1	k/d	1	B6§	b/b	7	0.09	0.05–0.19‡
	—		0	B6	b/b	7	0.02	0.01–0.08‡

SCID, +/+, and nude are as defined in Table I. All recipients in Exp. 1 were injected with poly(I:C) at the time of cell transfer.

* Inoculum size chosen such that splenic IUdR uptake values would fall on exponential portion of curve relating uptake with numbers of BMC grafted (16).

‡ Geometric mean value significantly ($p < 0.05$) lower than syngeneic controls.

§ Mice received 10^7 irradiated C3B6F1 (Exp. 1 and 2) or C3D2F1 (Exp. 3) SC and BMC intravenously at time of cell transfer of viable BMC.

the growth of B6 BMC. In Exp. 2, no poly(I:C) was used. The +/+ mice failed to resist 3.2×10^6 B6 BMC at this time, presumably because they are relatively poor responders to B6 (Hh-1^b) BMC grafts (1). However SCID mice resisted grafts of as much as 7.5×10^6 B6 BMC. This confirms earlier observations that SCID mice seem to display an augmented ability to reject marrow allografts as compared with their normal +/+ counterparts (7). Totally allogeneic C3B6F1 (H-2^k/H-2^b, Hh-1^{null}) BMC were not resisted by SCID, nude, or +/+ recipient mice (all H-2^d). These results do not support the self–nonself recognition hypothesis. However, they are consistent with the idea that H-2-heterozygous stem cells are lacking in the determinants, (presumably Hh) that are needed for NK cell–mediated marrow allograft rejection.

Immunization of Lethally Irradiated Mice to Reject H-2-Heterozygous Allogeneic BMC. The ability to immunize lethally irradiated rats to reject skin allografts within 7 d (12) suggested that we may be able to sensitize radioresistant T cells to reject murine BMC allografts. In the first experiment (Table II), lethally irradiated C.B-17 +/+ (H-2^d) mice were immunized (or not) with irradiated C3B6F1 SC and were simultaneously challenged with grafts of viable C3B6F1

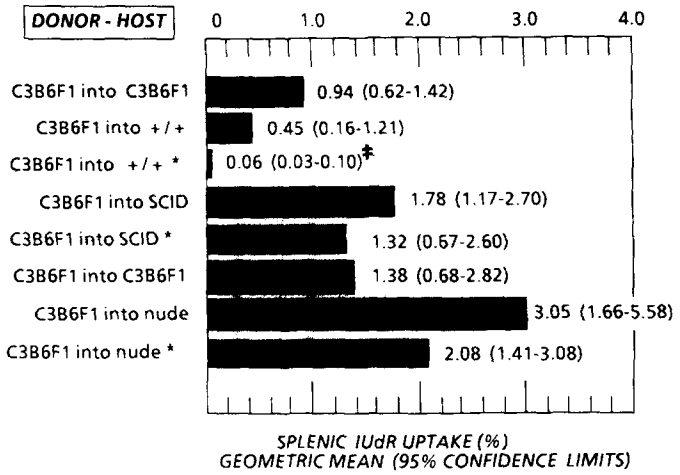

FIGURE 1. Effect of immunization on the growth of H-2-heterozygous allogeneic C3B6F1 (H-2^k/H-2^b, Hh-1^{null}) marrow grafts in C.B-17 *scid/scid* (SCID), BALB/c *nu/nu* (nude), and C.B-17 +/+ (+/+) (all H-2^d) recipients. Groups of six mice were lethally irradiated and infused with 10^6 viable C3B6F1 BMC with or without 10^7 irradiated C3B6F1 SC and BMC. The splenic ^{125}IUdR uptake assay was performed 7 d after cell transfer. *Hosts immunized with irradiated cells. ‡See Table II.

(H-2^k/H-2^b, Hh-1^{null}) BMC. Proliferation of the donor-derived BMC was measured 4 or 7 d after cell transfer. Only 10^6 BMC were grafted for the 7-d assay to prevent the isotope uptake values from falling off the exponential part of the dose-response curve. No resistance to grafted H-2 heterozygous C3B6F1 BMC was detected on day 4. In contrast, the immunized mice strongly resisted growth of C3B6F1 BMC by day 7 (Table II, Exp. *1b*). By day 7, even the unimmunized mice showed some resistance to engraftment. In the second experiment, the immunized C.B-17 +/+ hosts rejected C3B6F1 BMC by day 7, but not by day 4 (Table II, Exp. *2*). In a third experiment, using a different strain combination, B6 (H-2^b) hosts immunized with C3D2F1 irradiated cells were able to reject C3D2F1 (H-2^k/H-2^d, Hh-1^{null}) BMC by day 7 (Table II, Exp. 3). The kinetics of rejection suggest that the resistance to BMC grafts detected in the first 4 or 5 d after irradiation is mediated primarily by NK cells, but that T cells can certainly function by day 7.

Inability to Sensitize SCID or Nude Mice to Reject H-2-Heterozygous (Hh-1^{null}) Allogeneic BMC. The preceding data suggest that host T cells can be sensitized to H-2 alloantigens even after lethal total-body irradiation. If that is true, one should not be able to immunize SCID or nude mice, which lack T cell function. Therefore, C.B-17 +/+, SCID and nude (all H-2^d) mice were irradiated and infused with viable C3B6F1 BMC (H-2^k/H-2^b, Hh-1^{null}), and some hosts were simultaneously injected with irradiated C3B6F1 BMC and SC. Growth of the viable BMC was assessed 7 d after cell transfer. The +/+ mice, as before, rejected the H-2 heterozygous allogeneic BMC if immunized (Fig. 1). In contrast, the SCID and nude mice failed to reject the H-2 heterozygous allogeneic BMC even after immunization.

Surface Markers of the Effector Cells that Reject H-2-Homozygous vs. H-2-Hetero-

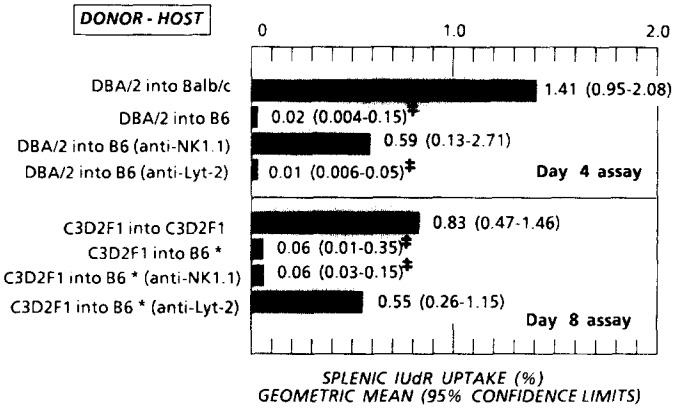

FIGURE 2. Effect of antibodies directed against NK or T cells to prevent rejection of BMC allografts. Groups of six B6 (H-2^b) mice were irradiated and infused with inocula of 2.5×10^6 DBA/2 (H-2^d, Hh-1^d) BMC or 10^6 C3D2F1 (H-2^k/H-2^d, H-1^{null}) BMC. *Hosts were immunized with irradiated (900 cGy) C3D2F1 BMC and SC. ‡Anti-NK-1.1 antibodies significantly ($p < 0.05$) enhanced growth of DBA/2 BMC in B6 hosts on day 4, while anti-Lyt-2 antibodies significantly ($p < 0.05$) enhanced growth of C3D2F1 BMC in immunized B6 hosts on day 8.

zygous Allogeneic BMC. To characterize the effector cells that reject H-2-homozygous (Hh-1^+) BMC at an early time (day 4) and the effector cells that, after immunization, reject H-2-heterozygous (Hh-1^-) BMC later (day 8), mAb to NK and T cell surface antigens were used. Preliminary experiments indicated that these antibodies removed the respective cell types, as determined by flow cytometry of lymphoid organs, even of unirradiated mice (data not shown). Prospective recipient B6 (H-2^b/Hh-1^b) mice were injected with anti-NK-1.1 or with anti-Lyt-2 reagents before irradiation and transfer of viable H-2 homozygous DBA/2 (H-2^d, Hh-1^d) or H-2 heterozygous C3D2F1 (H-2^k/H-2^d, Hh-1^{null}) BMC. The B6 mice challenged with C3D2F1 BMC were immunized with irradiated C3D2F1 BMC and SC at the time of transfer of viable BMC. Splenic ^{125}IUdR uptake was measured 4 d after transfer of DBA/2 BMC and 8 d after transfer of C3D2F1 BMC. The anti-NK-1.1 but not the anti-Lyt-2 antibodies prevented the rejection of H-2 homozygous DBA/2 BMC when growth was assessed on day 4 (Fig. 2). In contrast, the anti-Lyt-2 but not the anti-NK-1.1 antibodies prevented the rejection of H-2 heterozygous C3D2F1 BMC by the immunized B6 mice when growth was assessed on day 8 (Fig. 2). The data indicate that NK cells mediate rejection of H-2 homozygous allogeneic Hh-1^+ BMC while radioresistant Lyt-2^+ T cells mediate the rejection of H-2 heterozygous allogeneic Hh-1^- BMC.

Discussion

Model to Explain the Acute Rejection of H-2 Allogeneic BMC Grafts. The data presented here support the contention that two principal cell types are capable of rejecting H-2-incompatible BMC grafts in irradiated mice (Fig. 3). H-2-homozygous allogeneic stem cells express both Hh-1 and H-2 antigens, while H-2-heterozygous allogeneic stem cells express H-2 but not Hh-1 antigens. We suggest that NK cells have receptors for Hh-1 antigens but not H-2 antigens, and that Lyt-2^+ T cells (presumably cytolytic T lymphocytes) have receptors for

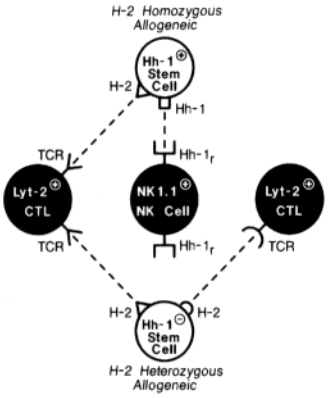

FIGURE 3. Model of potential effector mechanisms capable of mediating marrow allograft rejection after lethal irradiation. NK cells have receptors for Hh-1 antigens that are expressed on H-2-homozygous, but not on H-2-heterozygous, marrow stem cells. Lyt-2^+ T cells recognized conventional (presumably class I) H-2 antigens that are expressed on H-2-homozygous and H-2-heterozygous stem cells.

H-2 antigens and not Hh-1 antigens. Furthermore, NK cells act rapidly and early after challenge with Hh-1^+ cells, whereas T cell–mediated rejection of stem cells occurs later. Evidence to support these ideas is as follows: (*a*) SCID and nude mice, which display normal NK activity but are devoid of T cell functions, can reject BMC from allogeneic donors that are H-2-homozygous, but cannot reject BMC from donors that are H-2-heterozygous, even if they are immunized (Table I, Fig. 1); (*b*) rejection of H-2-homozygous allogeneic BMC by 4 d is mediated by NK-1.1^+ effector cells (NK cells) and not by Lyt-2^+ T cells (Fig. 2); (*c*) During the phase of NK cell–mediated rejection, irradiated and immunized mice cannot reject H-2-heterozygous (Hh-1^{null}) BMC by day 4 (Table II); (*d*) Immunization of irradiated mice at the time of BMC transfer results in the rejection of H-2-heterozygous allogeneic BMC by 7–8 d, which is mediated by Lyt-2^+ T cells and not by NK-1.1^+ cells (Table II and Fig. 2).

This model of rejection mechanisms may be able to explain previous apparently contradictory observations in the literature. For example, Lengerova and colleagues observed that F_1 hybrid BMC were rejected by parental-strain hosts (5), a finding at variance with the reported lack of immunogenicity of heterozygous stem cells (2). Moreover, Aizawa et al. (6) observed that F_1 hybrid BMC proliferated well for the first 5 d in parental-strain hosts, but were resisted at a later time by Thy-1.2^+ cells, presumably T cells. These findings can now be reconciled, since Lengerova used a splenic colony assay performed 9–10 d after BMC transfer, during which T cells reactive to H-2 alloantigens could have been generated. On the other hand, Bennett (2) used a 4-d ^{125}IUdR assay, at which time H-2-heterozygous BMC were never rejected but H-2-homozygous marrow allografts were, indicating that only NK cell–mediated rejection was occurring at this time point. Our model can also be used to reconcile and examine some of the issues currently involved with human bone marrow transplantation. The increased incidence of rejection of HLA allogeneic marrow grafts is associated with the detection of host-type T cells cytotoxic for donor-type cells in human marrow transplant recipients (14), and yet children with severe combined immunodeficiency (SCID) may resist BMC grafts if their NK cell function is nearly normal (21). It appears that, depending upon the circumstance (e.g., type of immunosuppression regimen used for the BMC transplant procedure), both of

the effector mechanisms examined with this murine model, i.e. NK and T cells, may play a role in the clinical outcome of human marrow transplant recipients.

Mechanism of Rejection of Marrow Cells by NK Cells. NK cells can mediate BMC graft rejection, but what is the mechanism of recognition? The ability of SCID mice devoid of B cells and immunoglobulins to reject H-2 homozygous allogeneic BMC suggests that NK cells do not have to use their Fc receptors for IgG to lyse marrow stem cells via antibody-dependent cellular cytotoxicity (22). Recent observations by Karre and colleagues have led to a new hypothesis for NK cell–mediated target cell lysis, with obvious extension to rejection of BMC grafts. The principal finding is that lymphoid tumor cells are much more susceptible to NK cells in vitro and in vivo if they express little or no class I H-2 antigens (9, 23, 24). Moreover, teratocarcinoma cells lose sensitivity to NK cells if they differentiate and thereby begin to express H-2 class I antigens (25, 26). Cytolytic T lymphocytes (CTL) recognize class I antigens on target cells and lyse those target cells that express high amounts of such antigens (27). Therefore it was postulated that T cells use their receptors to positively recognize MHC antigens on target cells, whereas NK cells are specifically triggered by the absence or reduced expression of self H-2 antigens. If this hypothesis were correct, one would expect that grafts from A × B donors into B hosts would be accepted, since B NK cells would recognize B H-2 antigens on A × B stem cells. In contrast, A × C marrow cells would be rejected by B hosts since B NK cells would be triggered to kill by the absence of B H-2 antigens on the A × C BMC. The inability of C.B-17 +/+ mice ($H\text{-}2^d$) to reject totally allogeneic C3B6F1 ($H\text{-}2^k/H\text{-}2^b$, $Hh\text{-}1^{null}$) BMC within 4 d even if boosted with poly(I:C), and the inability of B6 ($H\text{-}2^b$) mice to rapidly reject allogeneic C3D2F1 ($H\text{-}2^k/H\text{-}2^d$, $Hh\text{-}1^{null}$) BMC indicates that such self–nonself discrimination based on H-2 determinants does not apply to the NK cell–mediated rejection of BMC grafts.

Our data support the hypothesis that Hh-1 antigens are needed for the NK cell–mediated rejection of bone marrow allografts. This occurs, presumably, through the use of specific Hh-1 receptors. Unlike most other histocompatibility (i.e. class I) antigens, Hh-1 antigens are not inherited codominantly (2). A potential mechanism for the apparent recessive inheritance of Hh-1 antigens is that *trans*-acting genes downregulate the expression of Hh-1 antigens (28). This may explain why homozygosity at H-2 is required for optimal immunogenicity of BMC grafts. It should be noted that there is a limited polymorphism of Hh-1 antigens in the mouse, especially when compared with class I or class II antigens. Therefore, it is conceivable that instances of NK cell–mediated rejection of marrow grafts in humans (21) may be explained by recognition of Hh antigens, i.e. donors heterozygous for the highly polymorphic HLA class I and II determinants may still be homozygous for Hh determinants that are of limited polymorphism.

In conclusion, the data presented suggest that NK cells can mediate early BMC graft rejection, and only stem cells expressing Hh-1 antigens are recognized. At a later time, radioresistant T cells can mediate acute marrow graft rejection through the recognition of H-2 antigens, a process that is enhanced if the mice are immunized at the time of marrow transfer. Thus both NK and T cells can mediate acute marrow allograft rejection in lethally irradiated mice, but they

differ with respect to the target antigens recognized and the kinetics of the rejection response.

Summary

Lethally irradiated C.B-17 +/+, C.B-17 *scid/scid* (severe combined immunodeficiency, SCID), BALB/c-*nu/nu* (nude), and C57BL/6 (B6) mice were challenged with H-2-homozygous or H-2-heterozygous totally allogeneic bone marrow cell (BMC) grafts. Some of the irradiated mice were immunized simultaneously with large numbers of irradiated marrow and spleen cells syngeneic with the viable BMC transferred. Irradiated SCID and nude mice, devoid of T cells but with normal NK cell function, were able to reject H-2-homozygous BMC grafts within 4 d. However, they were unable to reject H-2-heterozygous BMC allografts by 7 d even if they were immunized. B6 and C.B-17 +/+ mice were able to reject H-2 heterozygous BMC allografts by 7–8 d, but not as early as 4 d, if they were immunized. The rejection of H-2-homozygous BMC on day 4 was inhibited by administration of anti-NK-1.1 antibodies, but not by anti-Lyt-2 antibodies. Conversely, the rejection of H-2-heterozygous allogeneic BMC on day 8 was prevented by anti-Lyt-2 but not by anti-NK-1.1 antibodies. The data indicate that both NK cells and Lyt-2^+ T cells can mediate rejection of allogeneic BMC acutely, even after exposure of mice to lethal doses of ionizing irradiation. NK cells appear to recognize Hemopoietic histocompatibility (Hh) antigens on H-2 homozygous stem cells. The inability of SCID and nude mice to reject H-2 heterozygous totally allogeneic BMC indicate that NK cells do not survey donor marrow cells for self H-2 antigens and reject those cells that express nonself H-2 antigens. The T cells presumably recognize conventional H-2 antigens (probably class I) under these conditions.

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