

## HUMORAL AND CELL-MEDIATED IMMUNE RESPONSES IN FULLY ALLOGENEIC BONE MARROW CHIMERA IN MICE\*

BY KAZUNORI ONOÉ, GABRIEL FERNANDES,‡ AND ROBERT A. GOOD

*From the Memorial Sloan-Kettering Cancer Center, New York 10021*

Recent studies with chimeras of hematopoietic and lymphoid system have revealed crucial restrictions on cellular interactions and immunologic functions attributable to the major histocompatibility complex (MHC)<sup>1</sup> (1-7). Most of this work has, thus far, used models employing semi-allogeneic chimeras or chimeras in which graft-vs.-host interactions were either ongoing or a real possibility (8, 9). This has been necessary because of the difficulty of establishing long-lived chimeras between histocompatible donors and recipients across major histocompatibility barriers. When insufficient immunosuppression (e.g., radiation) is given, the donor hematopoietic grafts are rejected by classical immunologic mechanism or do not take as a consequence of allogeneic resistance (10). When sufficient immunosuppression is given, graft-vs.-host reactions (GVHR) occur that are often lethal (9). Consequently, to date, only a few fully allogeneic chimeras have been studied (9, 11-17). As would be predicted from studies with the semi-allogeneic chimera (1, 2), these chimeras have shown evidence of varying degrees in immunoincompetence, but the studies are far from complete.

Advantage would accrue to analysis of MHC restrictions if healthy chimeras crossing major histocompatibility barriers could be produced and maintained over long periods in which the animals are entirely free of GVHR. Such animals would permit analysis of the influence of thymus and thymic epithelium in the absence of interactions with lymphohematopoietic cells histocompatible with donor cells, which is unavoidable in semi-allogeneic chimeras. Furthermore, under these circumstances, analysis of the influence of incompatibility of somatic cells with the lymphoreticular cells (3) could be examined *in vivo* without complicating consequences of GVHR.

The possibility of regularly achieving models of full chimerism that cross major histocompatibility barriers was demonstrated in rats by Müller-Ruchholtz et al. (14), who treated fatally irradiated rats with bone marrow of AgB (major histocompatibility system antigen)-incompatible donors, using bone marrow that had been exposed

---

\* Aided by grants from the National Institutes of Health (CA-08748, CA-17404, AI-11843, and NS-11457); The National Foundation-March of Dimes; the John A. Hartford Foundation; and the Judith Harris Selig Memorial Fund.

‡ To whom reprint requests should be addressed.

<sup>1</sup> *Abbreviations used in this paper:* ADCC, antibody-dependent cell-mediated cytotoxicity; BM, bone marrow; B6, C57BL/6; CI, cytotoxic index; Con A, concanavalin A; CRBC, chicken erythrocyte(s); CS, contact sensitivity; DNBS, 2,4-dinitrobenzene-1-sulfonic acid sodium salt; DNFB, 2,4-dinitro-1-fluorobenzene; EA, erythrocyte antibody complex; E<sup>k</sup>, B6-H-2<sup>k</sup> congenic mouse; FcR, Fc part of IgG; FCS, fetal calf serum; GVHR, graft-vs.-host reaction; H-2, murine major histocompatibility complex; LPS, lipopolysaccharide; MHC, major histocompatibility complex; NK, natural killer; PFC, plaque-forming cell; PHA, phytohemagglutinin; RL $\delta$ 1, radiation-induced leukemia in BALB/c strain; SI, spleen index; SRBC, sheep erythrocyte(s).

in vitro to anti-lymphocyte antiserum appropriately absorbed with both bone marrow and fetal liver cells to render it nontoxic to bone marrow stem cells. Long-lived, apparently immunologically vigorous, chimeras were achieved. Likewise, Truitt and Pollard (15) used germ-free donors and fatally irradiated germ-free recipient mice to create long-lived chimeras across major histocompatibility barriers. These animals have been found to be immunoincompetent in certain immunologic responses, but definition of these immunoincompetencies has been difficult because of uncertainty concerning the influence of GVHR or adjustments to GVHR.

Slavin et al. (16) have achieved a state of mixed chimerism using total nodal irradiation plus histoincompatible marrow transplantation. Reisner et al. (17) transplanted stem cells isolated from spleen, bone marrow, or thymus by agglutination and centrifugation after treatment in succession with soybean and peanut lectins and achieved long-lasting chimerism without producing GVHR. Thus far, in none of these models has precise composition of the chimeric state been defined or has comprehensive analysis of the immunocompetence of the chimeras been examined in light of the developing evidence of the restrictions imposed on immunologic functions by lack of histocompatibility of interacting cells (8, 9, 11-17).

It seems especially important to pursue investigations of fully allogeneic chimeras because approaches to adoptive immunotherapy (18) may benefit from such analyses. In addition, hematopoietic transplantation across major histocompatibility barriers in man offers attractive potentialities for cellular engineering to treat inborn or acquired errors of hematopoiesis and abnormalities of immunologic function (19, 20), and also offers the possibility of introducing resistance (R) genes for treatment and prevention of occurrence of leukemias, lymphoid malignancies, and, perhaps, other cancers (15, 21).

We report herein preparations of long-lived chimeras of AKR recipient and C57BL/6 (B6) donor mice using fatal total body irradiation plus injection of marrow that has been exposed in vitro to appropriate anti-Thy-1.2 antiserum in the absence of complement. When the marrow had been treated with this antiserum in vitro, virtually all the animals lived in good health for > 100 d after the transplant, and evidence of acute GVHR was not seen.

It is the purpose of this paper to present an analysis of the cellular makeup of the lymphoid tissues, and to evaluate hematopoietic and immunologic function of these radiation chimeras.

### Materials and Methods

*Animals.* Inbred AKR/J, B6, BALB/cJ, and C3H/HeJ were obtained from The Jackson Laboratory, Bar Harbor, Maine. B6-H-2<sup>k</sup> congenic mice were a gift from Dr. F. W. Shen, Memorial Sloan-Kettering Cancer Center. (B6 × AKR)<sub>F1</sub> and (B6 × BALB/c)<sub>F1</sub> mice were bred in our colony.

*Preparation of Bone Marrow Chimeras.* Unless stated otherwise, female AKR mice, 15-wk old, were used as irradiated recipients of marrow cells. The mice were irradiated in a plastic box with 900 rads using a <sup>137</sup>Cs- $\gamma$ -irradiation source at a dose rate of 125 rads/min. 24 h after irradiation, these mice were given an intravenous injection of  $25 \times 10^6$  B6 marrow cells treated in the following way: donor mice (female B6) were sacrificed by bleeding. Bone marrow cells were obtained from tibia and femur by gently flushing with cold RPMI-1640 supplemented with heat-inactivated fetal calf serum (FCS), 100 IU/ml penicillin, 100  $\mu$ g/ml streptomycin, and glutamine (Grand Island Biological Co., Grand Island, N. Y.). After centrifugation, marrow suspensions were adjusted to  $1 \times 10^8$ /ml and mixed with an equal volume of a 1:5 dilution of

a potent anti-Thy-1.2 serum. The cells were incubated with the antiserum on ice for 30 min with occasional mixing, washed three times with medium, and injected intravenously into lethally irradiated recipients. Complement was not added to this preparation, and the treatment did not cause death of the cells at this point because there was no change in trypan blue dye exclusion.

As controls,  $25 \times 10^6$  AKR syngeneic or  $25 \times 10^6$  unmanipulated B6 marrow cells were injected into the irradiated AKR recipients. In some experiments lethally irradiated AKR or B6 mice were reconstituted with B6-H-2<sup>k</sup> congenic mice (E<sup>k</sup>) or B6 marrow cells that had been treated with anti-Thy-1.2 serum alone, and irradiated B6 mice were given AKR marrow cells treated with anti-Thy-1.1 serum (1:5) without complement as described above.

All mice were given 400 mg/liter Terramycin (Pfizer Laboratories, Div. Pfizer Inc., New York) in their drinking water for 2 wk after irradiation. Thereafter, they were maintained on a conventional diet and given acidified water ad lib. The mice were observed daily for 100 d. For simplicity, chimeras prepared by injecting anti-Thy-1.2 serum-treated B6 marrow cells into irradiated AKR mice will be referred to as [B6 → AKR] chimeras; AKR mice treated with irradiation plus syngeneic cells as [AKR → AKR]; B6 mice treated with irradiation plus B6 cells as [B6 → B6]; B6 mice treated with irradiation plus AKR cells as [AKR → B6]; and AKR mice treated with irradiation plus E<sup>k</sup> cells as [E<sup>k</sup> → AKR].

*Antisera.* Anti-Thy-1.2 antiserum was prepared by injecting AKR/J female mice with  $5 \times 10^7$  C3H/HeJ thymus cells intraperitoneally at weekly intervals for 11 wk (22). To remove autoantibody, the serum was absorbed with AKR thymus cells before use. The limiting titer of the anti-Thy-1.2 antiserum that gave 50% lysis of B6 thymocytes was between 1/1,600 and 1/3,200, and the titer of anti-Thy-1.1 serum, which was provided by Dr. F. W. Shen, as described below, gave 50% lysis of AKR thymocytes at a titer of 1/1,280 and 1/2,560. The following antisera were used in typing for H-2 and Thy-1 phenotypes. Anti-Thy-1.1 ([B6 × A]F<sub>1</sub> anti-A-Thy-1.1 congenic mice thymocyte), anti-Thy-1.2 ([A-Thy-1.1 × AKR-H-2<sup>b</sup> congenic mice]F<sub>1</sub> anti-ASL1), anti-H-2<sup>b</sup> (E<sup>k</sup> anti-EL-4), and anti-H-2<sup>k</sup> (hybridoma: 5R3). The antisera used were kindly provided by Doctors F. W. Shen, E. Nakayama, N. Tada, and U. Hämmerling, Memorial Sloan-Kettering Cancer Center.

*Determination of Lymphoid Cell Chimerism.* All chimeras were maintained without further manipulation after hematopoietic reconstitution for 2.5–3.5 mo before analyses for chimerism and immunological functions. Chimerism was tested by analyzing spleen and thymus cells for susceptibility to cytotoxicity by the specific alloantisera mentioned above plus carefully selected rabbit or guinea pig complement. Typing for H-2 and Thy-1 surface antigens was performed by the cytotoxic test according to a modification of the method of Gorer and O’Gorman (23). Dilutions of antisera used in cytotoxic tests are indicated in Table I, and, in this condition, >95% of relevant target cells and <5% of negative control cells were killed. The percentage of dead cells was evaluated by counting labeled cells after addition of 0.2% trypan blue dye. The cytotoxic index (CI) was determined according to the following formula: CI equals

$$\frac{\text{percent killed with antiserum + complement} - \text{percent killed in complement control}}{100 - \text{percent killed in complement control}} \times 100.$$

The spleen cell preparations used in each immunological assay were analyzed for H-2 phenotype and in every instance the chimeric spleen cell preparations contained >95% cells of donor cell type.

*Erythrocyte Antibody Complex (EA)-Rosette-forming Cell Assay.* The proportion of the cells that possess a receptor for the Fc part of IgG (FcR) was determined by EA-rosette-forming cell assay as described previously (24). Briefly, sheep erythrocytes (E), sensitized with a subagglutinating dose of rabbit anti-sheep IgG (A) were mixed with spleen cells and incubated at 37°C for 5 min. After overnight incubation at 4°C, the pellet was gently resuspended and mixed with crystal violet solution (medium 199 that contained 1% glutaraldehyde and 2% crystal violet). Stained cells found with three or more EA by light microscopic examination at  $\times 100$  were considered rosettes. The results were expressed as percentages of rosettes in relation to the total number of nucleated cells.

*Simonsen’s Splenomegaly Assay.* The assay was performed according to the method by Simonsen (25). The spleens from B6, [B6 → AKR] chimeras, and (B6 × AKR)F<sub>1</sub> or (B6 × BALB/

c)F<sub>1</sub> were gently homogenized and the viable cell concentration adjusted to  $1 \times 10^8$ /ml in medium 199 that contained 2% FCS.  $1 \times 10^7$  spleen cells were then injected intraperitoneally into littermate 8-d-old (B6  $\times$  AKR)F<sub>1</sub> or (B6  $\times$  BALB/c)F<sub>1</sub> mice. 8 d later, both spleen-cell injected (experimental group) and medium injected (control group) were killed, their intact spleens removed and weighed, and the spleen index (SI) calculated (SI: spleen weight/body weight). GVHR index was detected according to the following formula: GVHR index = SI in experimental group/SI in control group. Control mice were thus given a GVHR index of 1.0.

To determine whether a suppressor cell mechanism participated in [B6  $\rightarrow$  AKR] chimeras,  $1 \times 10^7$  B6 or AKR spleen cells were mixed with  $5 \times 10^6$  [B6  $\rightarrow$  AKR] or (B6  $\times$  AKR)F<sub>1</sub> spleen cells and injected intraperitoneally into (B6  $\times$  AKR)F<sub>1</sub> littermate mice.

*Mitogen Stimulation.* Details of these assays have been described previously (26). Briefly,  $5 \times 10^5$  spleen cells were cultured with 0.25, 1.0, and 5.0  $\mu$ g of phytohemagglutinin (PHA) per milliliter (HA17, Burroughs Wellcome Co., Research Triangle Park, N. C.); 0.8, 2.5, and 5.0  $\mu$ g of concanavalin A (Con A) per milliliter (Sigma Chemical Co., St. Louis, Mo.); and 100  $\mu$ g of *Escherichia coli* lipopolysaccharide (LPS) per milliliter (Difco Laboratories, Detroit, Mich.) for 96 h. 16 h before harvest, 0.5  $\mu$ Ci of [*methyl*-<sup>3</sup>H]thymidine (NET-027, New England Nuclear, Boston, Mass.) was added to each culture well. The plates were harvested on glass-fiber filter papers, placed in scintillation fluid, and counted.

*Natural Killer (NK) Cell Activity and Antibody-dependent Cell-mediated Cytotoxicity (ADCC).* Spleens from each group of mice were collected in RPMI-1640 supplemented with 10% FCS (complete medium). The cells were released by gentle teasing in cold media, washed three times, and resuspended in complete medium to a concentration of  $20 \times 10^6$ /ml. Spleen cells from each group of mice to be compared were prepared similarly on the same day. RL $\delta$ 1 cells (radiation-induced leukemia in BALB/c strain) originally obtained from the National Institutes of Health (courtesy of Dr. R. Herberman) were used in NK assay. The NK activity was determined by a direct <sup>51</sup>Cr-release method as previously described (27).

The ADCC activity was determined by the method of Perlman as modified by Handwerker and Koren (28). Briefly, 50  $\mu$ l of varying concentrations of effector cells was added to 50  $\mu$ l of RPMI-1640 that contained  $10^4$  <sup>51</sup>Cr-labeled chicken erythrocytes (CRBC) and 100  $\mu$ l of a 1/10,000 final dilution of rabbit anti-CRBC antibodies. The plates were incubated for 3 h at 37°C in an atmosphere of 95% air and 5% CO<sub>2</sub>. The cytotoxicity was determined by a direct <sup>51</sup>Cr-release method.

*Response to In Vivo Immunization with Sheep Erythrocytes (SRBC).* Each mouse was injected intraperitoneally with 0.2 ml of a 10% suspension of SRBC (Grand Island Biological Co.). 4 d later the spleens were removed, gently homogenized, and the viable cell concentration adjusted to  $10^7$  cells/ml to measure primary immune response by the plaque-forming cell (PFC) assay previously described (29). To measure secondary immune response, mice were reimmunized 9 d after the first injection of SRBC and the PFC assay was carried out on day 13. To enumerate IgG PFC, rabbit anti-mouse IgG plus guinea pig complement was added in appropriate dilutions.

In one experiment, spleen cells were treated with anti-H-2<sup>k</sup> antibody or anti-H-2<sup>b</sup> serum and complement before incubating with SRBC on the culture plate (Table IV, footnote).

*Assay for Contact Sensitivity to 2,4-dinitro-1-fluorobenzene (DNFB) and Induction of Tolerance to DNFB.* DNFB was obtained from Sigma Chemical Co. 2,4-dinitrobenzene-1-sulfonic acid sodium salt (DNBS) was obtained from Eastman Kodak Co., Rochester, N. Y. Mice were sensitized by two daily applications of 25  $\mu$ l of 0.5% DNFB in 4:1 acetone:olive oil to the clipped skin of the abdominal wall (30). 4 d after the last painting, the thickness of both ears was measured with an engineer's micrometer as a base-line control. The mice were then challenged on the dorsal side of each ear lobe applying 20  $\mu$ l of 0.2% DNFB. Ear thickness was remeasured 24 h after challenge and expressed as the increase in thickness above base-line control value in units of  $10^{-3}$  cm  $\pm$  SD. Tolerance to DNFB was induced by intravenous injection of DNBS in sterile distilled water (750 mg/kg) 7 d before assay according to the method by Phanuphak et al. (30).

## Results

*Protection Against Lethal Irradiation by Allogeneic Marrow Transplantation with Marrow Cells Treated with Anti-Thy-1 Antiserum.* Fig. 1 presents survival curves that compare

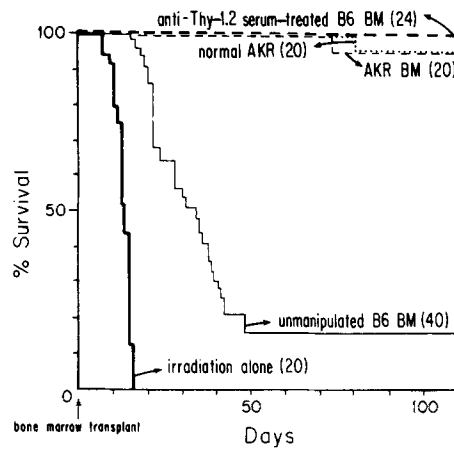


FIG. 1. Survival of chimeras after bone marrow (BM) transplantation. AKR recipient mice were lethally irradiated with 900 rads and reconstituted with  $25 \times 10^6$  AKR, nontreated B6, and anti-Thy-1.2 serum-treated B6 marrow cells. Data pooled from three separate experiments, show cumulative mortality after reconstitution with the BM cells. The number in parentheses represents the number of animals in each group. Normal AKR: age-matched nontreated AKR female mice.

3-mo-old female AKR mice treated by 900 rads total body radiation alone, 900 rads total body irradiation plus intravenous transplantation of  $25 \times 10^6$  marrow cells from 2- to 3-mo-old B6 female donors, 900 rads total body irradiation plus  $25 \times 10^6$  syngeneic AKR marrow cells, or  $25 \times 10^6$  marrow cells from B6 donors that had been exposed in vitro to anti-Thy-1.2 antiserum for 30 min before intravenous injection. It will be seen from the Fig. 1 that both AKR syngeneic marrow transplantation and transplantation of B6 marrow treated with anti-Thy-1.2 antiserum regularly permitted survival of AKR mice to 100 d. By contrast, 80% of AKR mice injected with B6 marrow cells after lethal total body irradiation, although surviving longer than irradiated mice, died within 50 d. 100% of mice given this dose of irradiation alone died within 15 d.

These findings show that injection of B6 marrow cells that have been treated with anti-Thy-1.2 antiserum without complement protects against lethal irradiation, does not induce GVHR, and permits prolonged survival of the lethally irradiated AKR mice.

*Chimerism by Allogeneic Marrow Transplantation after Treatment with Anti-Thy-1 Antiserum without Complement.* Table I presents results comparing representative observations concerning body weight, thymus weight, numbers of spleen cells, proportions of spleen, and thymus cells bearing H-2 and Thy-1 surface antigens and Fc receptors. It will be seen from the table that the [B6 → AKR] chimeric mice were generally well developed and healthy according to their body weight and by all parameters compared favorably with irradiated mice reconstituted with syngeneic marrow. The numbers of spleen cells recoverable from the allogeneic chimeras were slightly decreased as compared with irradiated mice reconstituted with syngeneic marrow and untreated control mice of the same age. Thymus weight also was well maintained in these allogeneic chimeras. Allogeneic chimeras prepared with bone marrow that had not been treated with anti-Thy-1 antiserum regularly had only a minisucle thymus or the thymus could not be found. It can also be seen from this table that the long-lived [B6 → AKR] allogeneic chimeric mice prepared as described are regularly full

TABLE I  
*Reconstitution of AKR Mice with Anti-Thy-1.2 Antiserum-treated B6 Bone Marrow Cells*

Group	Age	Age at transplant	Body		Spleen				Thymus					
			Weight	Index	Cell number	H-2 <sup>b</sup> (+) (1/80)	H-2 <sup>k</sup> (+) (1/200)	Thy-1.2 (+) (1/40)	FcR (+)	Weight	H-2 <sup>b</sup> (+) (1/40)	Thy-1.2 (+) (1/160)	Thy-1.1 (+) (1/160)*	
[B6 → AKR]	6	3	20.2	86¶	98	>95	**	‡‡	23	77	41	—	>95	0
			24.2	91	68	>95	—	—	20	68	63	—	>95	0
			25.0	102	73	>95	—	—	15	—	52	>95	>95	—
			23.5	103	128	>95	—	—	40	—	58	—	>95	—
			22.5	82	66	>95	—	—	—	—	—	—	—	—
			23.6	87	106	>95	0	—	—	—	—	—	—	—
						0								
[B6 → B6]‡	6	3	21.1	96	140	>95	—	22	—	—	—	>95	0	
[AKR → AKR]	6	3	26.5	98	102	<5	>95	—	—	—	—	0	>95	
B6§	6		23.1		106	>95	0	33	66	41	>95	>95	0	
AKR	6		27.6		92	<5	>90	0	61	57	<5	0	>95	

\* Dilution of antiserum used in cytotoxic test. See Materials and Methods.

‡ Syngeneic bone marrow transplantation.

§ Specificity control for antisera and complement used in the cytotoxic test.

|| Period after marrow transplantation.

¶ Body weight (BW) index = BW at assay/BW at bone marrow transplantation.

\*\* Cf. See Materials and Methods.

‡‡ Not done.

chimeras because >95% of their spleen cells or thymus cells were derived from the B6 donor marrow. By contrast, no cells of spleen or thymus could be shown to be attributable to the recipient's own cell lineages. In addition, the composition of the spleen cells shows slightly lower, but considerable, proportions of T cells, and these T cells were entirely of donor origin. These findings establish that it is possible to regularly prepare long-lived chimeras by fatal total body irradiation plus allogeneic bone marrow that has been treated with anti-Thy-1 antiserum. The chimeric mice prepared in this way remain in good health and do not show significant evidence of stress as would be revealed by thymic involution. The method further establishes a state of full hematopoietic and lymphoid chimerism and that these animals have considerable numbers of T lymphocytes.

*Capacity of Spleen Cells of Allogeneic Chimeras [B6 → AKR] to Initiate GVHR.* Fig. 2 summarizes data comparing GVHR initiated by spleen cells from [B6 → AKR] chimeras with GVHR initiated by B6 or (B6 × AKR)F<sub>1</sub> or (B6 × BALB/c)F<sub>1</sub> in Simonsen's spenomegaly assay (25) in (B6 × AKR)F<sub>1</sub> and (B6 × BALB/c)F<sub>1</sub> recipients. It will be seen from Fig. 2 that [B6 → AKR] chimeric mice do not initiate GVHR in (B6 × AKR)F<sub>1</sub> recipients (Panel a) but initiate as vigorous a GVHR in (B6 × BALB/c)F<sub>1</sub> recipients as do spleen cells from intact B6 donors (Panel b). These findings establish that the long-lived chimeric [B6 → AKR] mice that possess full B6 lymphoid chimerism are tolerant of both donor and recipient antigens and responsive to third party antigens represented in the BALB/c. There was a small but significant difference in GVHR activity between normal B6 and [B6 → AKR] chimera spleen cells when injected into (B6 × BALB/c)F<sub>1</sub> mice. This difference may also be reflected in a slightly lower proportion of T cells in the spleen cell suspension from the chimeras (Table I).

*Absence of Development of Specific Suppressor Phenomenon in the [B6 → AKR]*

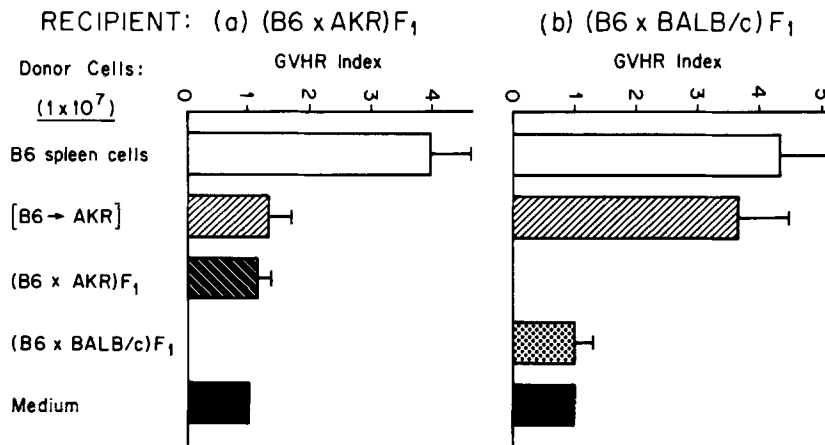


FIG. 2. Simonsen's splenomegaly assay.  $1 \times 10^7$  spleen cells from B6, [B6 → AKR] chimera, (B6 × AKR)F<sub>1</sub>, and medium were injected intraperitoneally into (B6 × AKR)F<sub>1</sub> (a), or  $1 \times 10^7$  cells from B6, [B6 → AKR], (B6 × BALB/c)F<sub>1</sub>, and medium into (B6 × BALB/c)F<sub>1</sub> littermate mice (b). The data show mean GVHR indices ± SD (Materials and Methods). *P* values from comparison of relevant experiment and control groups were ascertained by Student's *t* test. Significance of comparisons between B6, [B6 → AKR] chimera, and syngeneic F<sub>1</sub> spleen cells injected groups: (a) B6, *P* < 0.0005 for F<sub>1</sub>; B6, *P* < 0.0005 for [B6 → AKR]; all others nonsignificant. (b) B6, *P* < 0.0005 for F<sub>1</sub>; B6, 0.025 < *P* < 0.05 for [B6 → AKR]; [B6 → AKR], *P* < 0.0005 for F<sub>1</sub>; all others nonsignificant.

*Chimeras.* To determine whether the tolerant state in [B6 → AKR] chimeras is attributable to a suppressor cell mechanism, experiments were carried out in which spleen cells of [B6 → AKR] chimeras were mixed with either B6 or AKR lymphocytes and then injected into (B6 × AKR)F<sub>1</sub> recipients and evaluated in a Simonsen's assay 8 d later. It will be seen from Fig. 3 that the capacity of B6 or AKR spleen cells to initiate GVHR in (B6 × AKR)F<sub>1</sub> recipients is not inhibited by either the spleen cells of the [B6 → AKR] chimeras or spleen cells from (B6 × AKR)F<sub>1</sub> mice. We interpret these findings to establish that a suppressor cell phenomenon does not account for the tolerant state of the [B6 → AKR] chimeras spleen cells for either donor or recipient. The findings are compatible with the view that the failure of [B6 → AKR] chimeric cells to induce GVHR in the (B6 × AKR)F<sub>1</sub> recipient is attributable to classic immunologic tolerance or so-called tolerance by clonal depletion or inhibition (31) or immunologic unresponsiveness as exists in (B6 × AKR)F<sub>1</sub> self tolerance. The tolerant state under these circumstances cannot be attributed to a suppressor cell mechanism that involves spleen cells in the chimera.

*Responses of Spleen Cells of [B6 → AKR] Chimeras to Mitogens.* To evaluate the T-cell and B-cell reconstitution, proliferative responses of the [B6 → AKR] radiation chimeras induced by optimal concentrations of PHA, Con A, or LPS were studied. Representative experiments comparing responses to these mitogens of spleen cells from B6, or AKR mice, [B6 → AKR] chimeras and [AKR → AKR] and [B6 → B6] syngeneic marrow transplanted mice are compared (Table II). It will be seen from the table that although the proliferative responses to LPS or Con A were significantly lower for the [B6 → AKR] chimeras than those for AKR mice, they were comparable to those of mice reconstituted by syngeneic marrow transplantation or to B6 donor strains. These findings are interpreted as establishing that the [B6 → AKR] radiation chimeras have substantial populations of both T and B lymphocytes which can

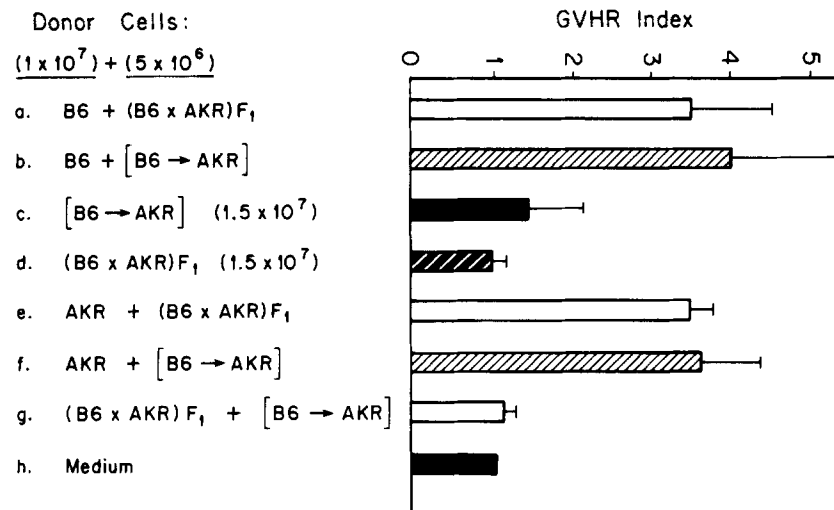
RECIPIENT: (B6 x AKR) F<sub>1</sub>

FIG. 3. No influence for GVHR indices of donor (B6) or recipient (AKR) spleen cells in (B6 x AKR) F<sub>1</sub> mice by adding the cells from [B6 → AKR] chimera.  $1 \times 10^7$  B6 or AKR spleen cells were mixed with  $5 \times 10^6$  [B6 → AKR] chimera or (B6 x AKR) F<sub>1</sub> spleen cells and injected intraperitoneally into (B6 x AKR) F<sub>1</sub> littermate mice (Fig. 2). In groups c and d,  $1.5 \times 10^7$  spleen cells from [B6 → AKR] chimeras and (B6 x AKR) F<sub>1</sub> mice were injected. Statistical comparisons of the GVHR indices of the various groups gave the following *P* values: a and c, a and d, b and c, and b and d, *P* < 0.005 in all cases; e and g, and e and h, *P* < 0.0005 in both cases; f and g, *P* < 0.005; and f and h, *P* < 0.01; all others nonsignificant.

respond by vigorous proliferation to stimulation with mitogens.

*NK Cell and ADCC Activities in [B6 → AKR] Chimeras.* On Table III are examined studies of NK and ADCC activities of spleen cells from AKR, B6, [B6 → B6] syngeneic marrow-reconstituted mice, and [B6 → AKR] chimeras. It will be seen from the table that the NK activities toward RL $\delta$ 1 cells and ADCC activities towards CRBC are comparable at two different effector:target cell ratios in mice of all four groups. These findings support the conclusion that the [B6 → AKR] chimeras do not differ from the donor or recipient strains or from syngeneically reconstituted mice according to these immunological parameters.

*PFC Responses to In Vivo Stimulation with SRBC in [B6 → AKR] Chimeras.* To evaluate capacity for T-cell-dependent antibody production the response to SRBC after primary antigenic stimulation or secondary stimulation were studied. The results of these experiments are summarized in Table IV. It will be seen from the table that the [B6 → AKR] chimeras were almost completely unresponsive to primary antigenic stimulation with SRBC as might be predicted from earlier experiments in other systems (1, 2, 5, 11-13). However, upon secondary stimulation the [B6 → AKR] chimeras showed a vigorous response in the direct (IgM) PFC assay and also developed surprisingly vigorous responses in the indirect PFC assay. The responses of the [B6 → AKR] chimeras in indirect plaque-forming assay were significantly lower than those of the AKR, B6, or [B6 → B6] mice. The direct PFC responses, on the other hand, were significantly greater than those of either AKR, B6, or [AKR → AKR] mice and equal to those of the [B6 → B6] mice after secondary stimulation.



TABLE II  
*Spleen Cell Responses to Mitogens in [B6 → AKR] Chimeras*

Group	Control*	Mitogen‡		
		PHA	Con A	LPS
AKR§ (4)	3,316 ± 703¶	59,914 ± 13,456	103,109 ± 10,900**	27,602 ± 3,197**
B6 (4)	1,297 ± 110	59,597 ± 3,264	80,190 ± 11,420‡‡	20,876 ± 2,459
[B6 → B6] (2)	1,905 ± 769	44,162 ± 3,347	42,533 ± 12,944	18,736 ± 3,453
[B6 → AKR] (5)	1,757 ± 507	51,161 ± 11,835	50,157 ± 12,442	20,570 ± 2,378

\* The background counts in cultures without mitogen.  
 ‡ PHA, 1.0 µg/ml; Con A, 2.5 µg/ml; LPS, 10 µg/ml.  
 § Age-matched nontreated mice.  
 || Number of animals in each group.  
 ¶ Data is expressed as the average counts per minute of [<sup>3</sup>H]Tdr ± SE.  
 \*\* Significantly higher than [B6 → AKR] (*P* < 0.01).  
 ‡‡ Not significantly higher than [B6 → AKR] (*P* > 0.05).

TABLE III  
*NK and ADCC Activity in [B6 → AKR] Chimeras*

Group	NK		ADCC	
	Percent specific lysis of			
	<sup>51</sup> Cr-RIΔ1		<sup>51</sup> Cr-CRBC	
	100:1	25:1	100:1	25:1
AKR (3)*	10.25 ± 2.65‡	3.51 ± 1.28	62.64 ± 4.09	35.21 ± 5.62
BT (4)	10.24 ± 3.77	4.96 ± 1.04	33.78 ± 0.03	15.36 ± 6.56
[B6 → B6] (2)	8.48 ± 1.33	2.47 ± 0.04	48.26 ± 7.24	35.44 ± 10.00
[B6 → AKR] (5)	7.20 ± 2.67§	2.65 ± 1.06	47.81 ± 12.16§	33.79 ± 13.31

\* Number of animals in each group.  
 ‡ Data are shown as mean ± SD.  
 § NK and ADCC activities of chimeras are not significantly different from those of normal AKR or B6 mice (*P* > 0.05).

To determine whether the secondary PFC responses in the [B6 → AKR] chimeras were reflecting an influence of AKR cells, the responses were compared in unmanipulated spleen cells and in spleen cells treated with anti-H-2<sup>k</sup> or anti-H-2<sup>b</sup> antibody plus complement. Such cytotoxic antibody treatment would eliminate all AKR cells or, alternatively, cells of the B6 donor origin (Table IV, footnote). The anti-H-2<sup>k</sup> treatment failed completely to alter the capacity of the spleen cells of the chimeric mice to produce either direct or indirect plaques in the secondary response. By contrast, treatment with anti-H-2<sup>b</sup> antiserum almost completely inhibited capacity to produce direct PFC and very much reduced capacity to form indirect PFC (Table IV).

*Primary Responses to SRBC of Additional H-2-Compatible and -Incompatible Combination.* To determine whether the results presented in the previous section showing complete failure of primary immune response in [B6 → AKR] irradiation chimeras were a more general case and based upon genetic differences of donor and recipient at MHC, an additional set of experiments were performed. After lethal total body irradiation, mice of AKR or B6 strains were reconstituted by treatment with

TABLE IV  
Anti-SRBC PFC Response in Chimeras\*

Group	Direct PFC per spleen	Indirect PFC per spleen
Primary‡		
AKR (10)	50,295 ± 9,004¶	57,762 ± 10,298
B6 (10)	39,488 ± 14,372	35,122 ± 11,402
[AKR → AKR] (5)	62,634 ± 3,610	54,697 ± 7,177
[B6 → AKR] (9)	156 ± 137**	256 ± 174‡‡
Secondary§		
AKR (4)	1,489 ± 630	89,640 ± 10,688
B6 (5)	5,733 ± 1,211	106,862 ± 34,469
[AKR → AKR] (3)	4,457 ± 2,385	94,147 ± 43,214
[B6 → B6] (6)	13,856 ± 3,747	170,515 ± 21,385
[B6 → AKR] (9)	15,626 ± 3,018§§	50,889 ± 8,637
[B6 → AKR] (4)		
nontreated	14,412 ± 1,886	56,753 ± 19,889
Treated with¶¶ anti-H-2 <sup>k</sup> and complement	12,996 ± 5,566	56,697 ± 25,621
treated with*** anti-H-2 <sup>b</sup> and complement	167 ± 27	5,154 ± 1,730

\* Data pooled from three separate experiments.

‡ 4 d after immunization with 0.2 ml of 10% SRBC.

§ 4 d after reimmunization, which was given 9 d after primary immunization.

|| Number of animals in each group.

¶ Mean ± SE.

\*\* Significantly lower than any other groups,  $P < 0.01$ .

‡‡ Significantly lower than any other groups,  $P < 0.025$ .

§§ Significantly higher than AKR, B6, and [AKR → AKR],  $P < 0.025$ .

||| Significantly lower than AKR, B6, and [B6 → B6],  $P < 0.05$ .

¶¶  $1 \times 10^7$ /ml spleen cells were incubated with the same volume of (1:200) anti-H-2<sup>k</sup> antibody and (1:8) selected rabbit complement at 37°C for 45 min. After washing three times, cells were assayed for PFC. The number of PFC of normal AKR spleen cells was suppressed 98% (direct) and 76% (indirect) by this treatment.

\*\*\*  $1 \times 10^7$ /ml spleen cells were incubated with the same volume of (1:40) anti-H-2<sup>b</sup> antiserum and (1:4) guinea pig complement. The number of PFC of normal B6 spleen cells was suppressed 99% (direct) and 70% (indirect) by this treatment.

bone marrow from AKR or B6 donors that had been treated in vitro with anti-Thy-1 serum ([AKR → AKR], [AKR → B6], [B6 → AKR]). Alternatively, lethally irradiated AKR mice were treated with anti-Thy-1 serum-treated marrow from E<sup>k</sup> congenic mice, which are B6 background but have H-2<sup>k</sup> haplotype instead of H-2<sup>b</sup> (E<sup>k</sup> → AKR). 80 d after successful bone marrow transplantation that did not produce evidence of acute GVHR, the mice were stimulated with SRBC. PFC responses of spleen cells were analyzed 4 d later. The [E<sup>k</sup> → AKR] H-2-compatible chimeric mice were regularly shown to be full chimeras because their thymus cells were entirely of donor origin (Table V, footnote).

The results summarized in Table V show conclusively that in each instance when the bone marrow cells are from donors histocompatible with the recipients at the H-2 locus, the primary immune response shows numbers of both direct and indirect plaques equal to or approaching those of the unmanipulated B6 or AKR controls. In every instance where donor and recipient were incompatible at the H-2 locus, both

TABLE V  
Primary Anti-SRBC PFC Response in H-2-compatible Chimeras\*

Group	Direct PFC per spleen	Indirect PFC per spleen
AKR (3)‡	80,980 ± 20,944	34,935 ± 5,989
B6 (3)	33,824 ± 7,787	12,928 ± 5,990
[AKR → AKR] (4)	44,817 ± 4,916	18,651 ± 3,809
[AKR → B6]§ (2)	232 ± 88	20 ± 20
[B6 → AKR] (3)	97 ± 72	0 ± 0
[E <sup>kl</sup> → AKR] (3)	31,543 ± 4,171¶	11,714 ± 4,482¶

\* 4 d after immunization with 0.2 ml of 10% SRBC.

‡ See Table IV, footnote ||.

§ 25 × 10<sup>6</sup> anti-Thy-1.1 antiserum (1:5) treated AKR bone marrow cells transplanted into lethally irradiated (900 rads) B6 mice. Spleen cells from both chimeras showed >90% H-2<sup>k</sup> (+).

|| B6-H-2<sup>k</sup> congenic mice. Chimerism tested by analyzing only thymocytes, because anti-H-2 serum was unavailable in this combination. Each chimera showed Thy-1.2 (+) cells 93, > 95, and > 95%; Thy-1.1(+) cells 0, < 5, and < 5%, respectively.

¶ Significantly higher than [AKR → B6] and [B6 → AKR], P < 0.01.

TABLE VI  
CS and Tolerance Induction to DNFB in Chimeras\*

Group	DNBS	DNFB	Ear swelling cm <sup>-3</sup>
AKR			
(6)‡	-	-	1.29 ± 0.89§
(8)	-	+	12.06 ± 2.30
(6)	+	+	1.88 ± 1.28
[AKR → AKR]			
(5)	-	+	8.15 ± 2.89
(5)	+	+	1.45 ± 0.86
B6			
(7)	-	-	2.07 ± 1.17
(6)	-	+	8.96 ± 1.88
(6)	+	+	1.46 ± 0.94
[B6 → B6]			
(3)	-	+	9.50 ± 2.12
(3)	+	+	1.50 ± 0.71
[B6 → AKR]			
(6)	-	-	1.81 ± 1.26
(6)	-	+	9.27 ± 4.20
(6)	+	+	9.21 ± 3.26
[E <sup>kl</sup> → AKR]			
(4)	-	+	7.19 ± 2.83
(3)	+	+	1.17 ± 0.68

\* Data pooled from two separate experiments.

‡ The number in parentheses represents the number of animals in each group.

§ Mean ± SD.

|| See Table V, footnote ||.

direct and indirect plaque-forming responses were negligible. These findings supplement those of the prior experiment in establishing that in full chimeras the primary antibody response to thymus-dependent SRBC antigen requires histocompatibility at MHC between donor and recipient.

*Contact Sensitivity and Specific Unresponsiveness to Contact Sensitivity in [B6 → AKR] Chimeras.* To investigate interactions of the immunocompetent cells and the host cells or cellular components, an analysis was carried out of the capacity of the [B6 → AKR] chimeras to develop and express contact sensitivity (CS) to DNFB, using the ear swelling technique of Phanuphak et al. (30). Representative data are presented on Table VI. It can be seen from the table that vigorous capacity to develop and express CS was present in the long-lived [B6 → AKR] mice. Indeed this form of cell-mediated immunity was as vigorous in these chimeras as in AKR, B6, [AKR → AKR], [B6 → B6], or [E<sup>k</sup> → AKR] mice.

Analyses to evaluate capacity to produce specific tolerance to DNFB by intravenous administration of DNBS before the sensitizing application of DNFB gave different results. The [B6 → AKR] chimeras appeared to be completely unable to develop the specific unresponsiveness to stimulation with DNFB by the intravenous route. By contrast, irradiated B6 mice or AKR mice reconstituted with syngeneic marrow cells ([B6 → B6], [AKR → AKR]) or irradiated AKR mice reconstituted with H-2-compatible marrow cells ([E<sup>k</sup> → AKR]) developed specific unresponsiveness to the sensitization by DNFB applied to the skin as did the mice of B6 and AKR donor and recipient strains. These findings establish that [B6 → AKR] mice respond poorly to this form of tolerance induction, desensitization, or immunization.

### Discussion

In these studies we present a reproducible method for preparing long-lived allogeneic chimeras where donor and recipient mice differ from one another across the major histocompatibility barrier, H-2. In the strain combination used, B6 donor and AKR recipient, which represents a strain combination with strong hybrid resistance to bone marrow transplantation (10), the allogeneic chimeras [B6 → AKR] showed no evidence of GVHR when the marrow cells had been treated in vitro before injection by a potent anti-Thy-1.2 antisera in the absence of complement. By contrast, control mice given allogeneic marrow not so treated regularly survived longer than irradiated controls, but most of them died before 50 d with evidence of GVHR. From studies of the thymus and spleen cells it could be shown that these chimeric AKR mice possessed virtually all B6 (H-2<sup>b</sup>) lymphocytes but no cells with AKR (H-2<sup>k</sup>) surface antigens. The Thy-1.2 antigen of the B6 donor T-cell type was present on the thymus cells, and an appropriate portion of the spleen cells and no evidence of Thy-1.1 (+) cells of AKR recipient origin could be demonstrated. Thus these were full chimeras. The [B6 → AKR] chimeras were shown to be tolerant of donor and recipient alloantigens by the Simonsen assay (25) and nearly fully reactive to third party alloantigens as revealed by the ability of their spleen cells to produce splenomegaly in (B6 × BALB/c)F<sub>1</sub> recipients. However, a slightly diminished response toward third party antigens was seen (Fig. 2), which may have been attributed to a small deficiency of T-cell numbers in the spleen (Table I).

Interestingly, the tolerance observed appeared to be of classic type, probably based on clonal inhibition or deletion (31) and not on a suppressor cell mechanism because

the spleen cells of these chimeras did not suppress the ability of the normal spleen cells from B6 or AKR donors to initiate GVHR in (B6 × AKR) $F_1$  mice. To establish the absence of suppressor cells convincingly, further studies in search of suppressor cells function may be necessary in experiments that use higher proportions of the spleen cells from the chimeras, because in the analysis carried out here, only one ratio of the mixture of spleen cells from the chimera and normal B6 or AKR mice was used and a higher ratio might show evidence of suppressor cells, as reported by Urso and Gengozian (32). In addition, other kinds of analyses (e.g., mixed lymphocytes reaction, cell-mediated lympholysis [33]) of suppressor influence should also be carried out.

Spleen cells from these fully allogeneic chimeras were also able to demonstrate vigorous proliferative responses to PHA, Con A, and LPS stimulations *in vitro*. Thus, they had well-developed populations of both T and B lymphocytes that showed little or no evidence of being suppressed. Because some of these responses require T cell-T cell interactions (34) or interactions of T cells with cells of monocyte-macrophage lineage (35), these chimeric mice appeared to have interaction of monocyte-macrophage lineage and the T lymphocytes sufficient to permit normal proliferative response to Con A, PHA, and LPS. This is, however, a somewhat controversial issue since McCombs et al. (36) reported that histocompatibility between lymphocyte and macrophage is not a requirement for proliferative responses to mitogens. NK and ADCC functions of these chimeric mice were also in normal range.

Studies of the PFC responses to stimulation with SRBC are of special interest. No direct or indirect PFC responses were observed upon primary stimulation. This limitation was seen in [B6 → AKR] and [AKR → B6] chimeras, whereas it was not seen with [AKR → AKR] preparations or when  $E^k$  congenic mice were the donors [ $E^k$  → AKR] (Table V).

Thus, the primary responses in these fully chimeric mice where mature T cells have been eliminated from the precursors used for immunologic and hematopoietic reconstruction are deficient in T-dependent antibody formation as might be predicted from studies of genetic restriction previously reported (1, 2, 5, 37). Nonetheless, strikingly vigorous responses of antibody-forming cells to the T-dependent antigen were observed for both IgM- and IgG-type plaque formation during a secondary response in the [B6 → AKR] chimera (Table IV). The indirect plaque formation was somewhat lower and the direct PFC responses considerably higher than were seen with the B6, AKR, or [AKR → AKR] controls. However, even the mice reconstituted after lethal total body irradiation by syngeneic marrow cells showed in the secondary response somewhat higher IgM plaques than did either donor or recipient strain. Thus, it would appear that although some degree of allogeneic preference or restriction in indirect PFC response may be obtained in these chimeric mice, it is not as limiting with respect to secondary T-dependent antibody responses as might be extrapolated from the existing literature (1-3, 5, 6, 11-13, 37-39). It seems to us that these findings are concordant with prior observations by Gengozian et al. (40), von Boehmer et al. (41), Erb et al. (42), Hong et al. (43), and Katz et al. (44), which suggest that the restrictions are not absolutely binding if there is opportunity for prior experience and a period for learning.

The studies of CS in these fully allogeneic chimeras were also most fascinating. Skin reactions after sensitization with DNFB were not different from those of normal mice or of irradiated mice reconstituted with syngeneic bone marrow cells. This was

somewhat surprising since Miller et al. (45) showed that identity at the MHC is necessary for passive transfer of delayed type hypersensitivity to DNFB and Asherson et al. (46) reported that active immunization with a small number of lymph node cells from donors immunized with contact sensitizer (oxazolone) 24 h before injection occurred only when a genetic matching at MHC between donor and recipient mice existed. These results, however, are also somewhat controversial, and our findings clearly indicate that vigorous CS to DNFB can occur when a state of full chimerism exists in which the lymphoid cells are of donor origin and the skin cells of host origin. Recent evidence suggests that Langerhans cells of the skin may play an important role in the development of CS because they can absorb certain antigens and possess Ia antigens and Fc receptors (47-49). It thus seems important to know if in these chimeras the Langerhans cells are of donor or host origin. This should be studied.

By contrast to the observations with CS, desensitization, or tolerization by intravenous injection of DNBS was ineffectual in preventing CS to DNFB in these allogeneic chimeras but was fully effective in the lethally irradiated mice reconstituted with syngeneic or H-2-compatible marrow cells. Whether or not the rather complex tolerance in intact animals, which is known to depend in part on T-suppressor cells (50), augmenting cells for the T-suppressor cells (51), and even classical clonal inhibition (50, 52) can be developed in fully allogeneic chimeras by repeated stimulation with DNBS remains to be determined in future studies.

Because our experiments appear to lend an optimistic perspective to the possibility of cellular engineering (19) employing allogeneic cells differing from host across major histocompatibility barrier it will be of interest to know whether, in this model and in those employed by Müller-Ruchholtz et al. (14) with rats, or that of Truitt and Pollard (15) with mice, truly long-lived [B6 → AKR] mice can be obtained that can resist bacterial and viral infections normally. Experiments to address these important questions with fully allogeneic chimeras seem in order. One would surely predict from the most recent experiments of Zinkernagel et al. (2, 3)<sup>2</sup> that the T lymphocyte developing in contact with the thymus epithelium of the recipients would be able to address the host-virus-infected somatic cells with normal immunologic vigor. It will be especially important to know whether allogeneic cells prepared with antiserum to eliminate T cells from marrow after a gentle manipulation, such as was used here, can resist the malignancies caused by virus or chemical carcinogens. The AKR recipients thus seem to be an especially good model for further inquiries into the effectiveness of the cellular and immunologic reconstitution after lethal irradiation and bone marrow transplantation made possible by treatment to eliminate post thymic cells with anti-Thy-1 serum *in vitro*.

Whether similar results will be obtained with other strain combinations in mice remains to be seen. Additional investigations in our own laboratories establish that long-lived, immunologically vigorous chimeras B6 → CBA may be produced if the marrow cells are pretreated with appropriate anti-Thy-1 plus complement (S. Krown, R. Colco, G. Fernandes, and R. A. Good. Unpublished data.) This is the same genetic barrier studied in our experiments and is usually considered to be among the most resistant to allogeneic transplantation. Whether the preparatory treatment with anti-

<sup>2</sup> Zinkernagel, R. M., and P. C. Doherty. MHC-restricted cytotoxic T cells: studies on the biological role of the polymorphic major transplantation antigens determining T-cell restriction-specificity function and responsiveness. Manuscript in press.

Thy-1 without complement has advantage over the preparatory treatment with anti-Thy-1 plus complement will have to be ascertained by direct comparative analyses of the two methods. That pretreatment with anti-Thy-1 serum without complement is effective, as shown herein, is of great importance because so many of the sources of complement one would like to use are also cytotoxic to the stem cells that one does not wish to damage.

### Summary

AKR mice were protected from lethal irradiation and established as long-lived chimeras by transplanting allogeneic C57BL/6 (B6) bone marrow that had been treated in vitro with anti-Thy-1 antiserum without complement. In these chimeras, which were designated [B6 → AKR], virtually all the thymus and spleen cells were shown to be derived from the B6 donor; several immune functions studied in these chimeras were as follows: (a) The chimeric mice were tolerant of histocompatibility antigens of both donor and recipient strain and nearly fully reactive to antigens of third party, as revealed by Simonsen's splenomegaly assay. The tolerance of these chimeras could not be attributed to suppressor cells but was compatible with clonal depletion. (b) Proliferative responses to concanavalin A, phytohemagglutinin, and lipopolysaccharide as well as natural killer and antibody-dependent cell-mediated cytotoxicity activity of the chimeric mice was normal. (c) Plaque-forming cell (PFC) assays of antibody responses to sheep erythrocytes (SRBC) showed gross deficiency in the primary response of the [B6 → AKR] and [AKR → B6] chimeras.

By contrast, [B6-H-2<sup>k</sup>(E<sup>k</sup>) → AKR] H-2-compatible chimeras and [AKR → AKR] syngeneic marrow transplanted mice had normal primary PFC responses. PFC responses after secondary stimulation with SRBC, however, revealed vigorous direct plaque formation and substantial but somewhat smaller indirect plaque formation in the [B6 → AKR] chimeras. This observation favors operationally the concept of adaptive differentiation proposed by Katz et al. (44). (d) Analysis of ability of the chimeras to develop and express delayed-type hypersensitivity responses to contact sensitizer (2,4-dinitro-1-fluorobenzene [DNFB]) showed no apparent immunodeficiency of either chimeras to this form of immunization. Development of immunologic tolerance to DNFB, however, was grossly deficient in [B6 → AKR] chimeras but normal in [AKR → AKR], [B6 → B6], and [E<sup>k</sup> → AKR] chimeras.

These findings indicate that full chimeras across major histocompatibility complex have considerable immunologic vigor even though primary immune responses that require histocompatibility between interacting cell types are initially defective.

The authors gratefully acknowledge the skillful technical assistance of Gerald Vogel, Fred Barreau, and Glenda McFeeters.

*Received for publication 14 August 1979.*

### References

1. Bevan, M. 1977. In a radiation chimera, host H-2 antigens determine immune responsiveness of donor cytotoxic cells. *Nature (Lond.)* **269**:417.
2. Zinkernagel, R. M., G. N. Callahan, A. Althage, S. Cooper, P. A. Klein, and J. Klein. 1978. On the thymus in the differentiation of "H-2 self-recognition" by T cells: evidence for dual recognition? *J. Exp. Med.* **147**:882.

3. Zinkernagel, R. M., G. N. Callahan, A. Althage, S. Cooper, J. W. Streilein, and J. Klein. 1978. The lymphoreticular system in triggering virus plus self-specific cytotoxic T cells: evidence for T help. *J. Exp. Med.* **147**:897.
4. Katz, D. H., B. J. Skidmore, L. R. Katz, and C. A. Bogowitz. 1978. Adaptive differentiation of murine lymphocytes I. Both T and B lymphocytes differentiating in  $F_1 \rightarrow$  parental chimeras manifest preferential cooperative activity for partner lymphocytes derived from the same parental type corresponding to the chimeric host. *J. Exp. Med.* **148**:727.
5. Sprent, J., and H. von Boehmer. 1979. T-helper function of parent  $\rightarrow F_1$  chimeras, presence of a separate T-cell subgroup able to stimulate allogeneic B cells but not syngeneic B cells. *J. Exp. Med.* **149**:387.
6. Erb, P., B. Meier, D. Kraus, H. von Boehmer, and M. Feldman. 1978. Nature of T cell-macrophage interaction in helper cell induction in vitro. I. Evidence for genetic restriction of T cell-macrophage interactions prior to T cell priming. *Eur. J. Immunol.* **8**:786.
7. Vadas, M. A., J. F. A. P. Miller, A. M. Whitelaw, and J. P. Gamble. 1977. Regulation by the H-2 gene complex of delayed type hypersensitivity. *Immunogenetics.* **4**:137.
8. Gengozian, N., and P. Urso. 1976. Status of T- and B-cell cooperation in radiation chimeras: evidence for a suppressor effect. *Transplant. Proc.* **8**:631.
9. Mattingly, J. A., and P. M. Webb. 1978. Studies on the mitogen responses of germ free allogeneic chimeras. II. Maturation of two cell types and partial restoration of responsiveness of the short-term chimeras. *J. Immunol.* **120**:1274.
10. Cudkowicz, G., and M. Bennett. 1971. Peculiar immunobiology of bone marrow allografts. II. Rejection of parental grafts by resistant  $F_1$  hybrid mice. *J. Exp. Med.* **134**:1513.
11. Urso, P., and N. Gengozian. 1974. Variation in T and B cell deficiency in different mouse allogeneic radiation chimeras. *J. Immunol.* **113**:1770.
12. Nordin, A. A., and J. J. Farrar. 1974. Studies of the immunological capacity of germfree mouse radiation chimeras. III. In vitro reconstitution of the T-helper cell deficiency. *Cell. Immunol.* **10**:218.
13. Trier, L., and B. Rubin. 1974. Bone marrow transplantation in inbred strain of mice. I. The failure of development of normal T cell function following allogeneic transplantation. *Acta Pathol. Microbiol. Scand. Sect. B. Microbiol. Immunol.* **82**:724.
14. Müller-Ruchholtz, W., H. U. Wottge, and H. K. Müller-Hermelink. 1976. Bone marrow transplantation in rats across strong histocompatibility barriers by selective elimination of lymphoid cells in donor marrow. *Transplant. Proc.* **8**:537.
15. Truitt, R. L., and M. Pollard. 1976. Allogeneic bone marrow chimerism in germ-free mice. IV. Therapy of "Hodgkin's-like" reticulum cell sarcoma in SJL mice. *Transplantation (Baltimore)*. **21**:12.
16. Slavin, S., B. Reitz, C. P. Bieber, H. S. Kaplan, and S. Strober. 1978. Transplantation tolerance in adult rats using total lymphoid irradiation: permanent survival of skin, heart, and marrow allografts. *J. Exp. Med.* **147**:700.
17. Reisner, Y., L. Itzicovitch, A. Meshorer, and N. Sharon. 1978. Hemopoietic stem cell transplantation using mouse bone marrow and spleen cells fractionated by lectins. *Proc. Natl. Acad. Sci. U. S. A.* **75**:2933.
18. Mathé, G., L. Schwarzenberg, O. Halle-Pannenko, N. Abuaf, and P. Pouillart. 1977. Of mice and men in bone marrow transplantation. *Transplant. Proc.* **9**:155.
19. Good, R. A. 1977. Cellular engineering. *Clin. Bull. (Mem. Sloan-Kettering Cancer Cent.)*. **7**:33.
20. Hansen, J. A., R. J. O'Reilly, R. A. Good, and B. Dupont. 1976. Relevance of major human histocompatibility determinants in clinical bone marrow transplantation. *Transplant. Proc.* **8**:581.
21. Tanaka, T., Y. Obata, G. Fernandes, K. Onoé, E. Stockert, and R. A. Good. 1979. Prevention of leukemia in lethally irradiated AKR mice by CBA/H marrow transplantation. *Proc. Am. Assoc. Cancer Res.* **20**:114. (Abstr.)
22. Korngold, R., and J. Sprent. 1978. Lethal graft-versus-host disease after bone marrow



- transplantation across minor histocompatibility barriers in mice. *J. Exp. Med.* **148**:1687.
23. Gorer, P. A., and P. O'Gorman. 1956. The cytotoxic activity of isoantibodies in mice. *Transplant. Bull.* **3**:142.
  24. Onoé, K., R. Yasumizu, J. Takeda, H. Okuyama, and K. Morikawa. 1979. The study of difference in the proportions of FcR lymphocytes between the spleen and the lymph node. *Bull. Inst. Immunol. Sci. Hokkaido Univ.* **39**:1.
  25. Simonsen, M. 1962. Graft versus host reactions. Their natural history and applicability as tools of research. *Prog. Allergy.* **6**:349.
  26. Fernandes, G., B. H. Handwerger, E. J. Yunis, and D. M. Brown. 1978. Immune response in the mutant diabetic C57BL/Ks-db-mouse. Discrepancies between in vitro and in vivo immunological assays. *J. Clin. Invest.* **61**:243.
  27. Herberman, R. B., M. E. Nunn, H. T. Holden, and D. H. Lavrin. 1975. Natural cytotoxic reactivity of mouse lymphoid cells against syngeneic and allogeneic tumors. II. Characterization of effector cells. *Int. J. Cancer.* **16**:230.
  28. Handwerger, B. S., and H. S. Koren. 1976. The nature of the effector cell in antibody-dependent cell-mediated cytotoxicity (ADCC): the cytotoxic activity of murine tumor cells and peritoneal macrophages. *Clin. Immunol. Immunopathol.* **5**:272.
  29. Fernandes, G., M. Nair, K. Onoé, T. Tanaka, R. Floyd, and R. A. Good. 1979. Impairment of cell-mediated immunity functions by dietary Zinc deficiency in mice. *Proc. Natl. Acad. Sci. U. S. A.* **76**:457.
  30. Phanuphak, P., J. W. Moorhead, and H. N. Claman. 1974. Tolerance and contact sensitivity to DNFB in mice. I. In vivo detection by ear swelling and correlation with in vitro cell stimulation. *J. Immunol.* **112**:115.
  31. Burnet, F. M. 1959. *The Clonal Selection Theory of Acquired Immunity.* Cambridge University Press, New York.
  32. Urso, P., and N. Gengozian. 1978. Suppression of GvH and PFC responses by spleen cells from radiation chimeras. *Fed. Proc.* **37**:1269 (Abstr.)
  33. Sprent, J., H. von Boehmer, and N. Nabholz. 1975. Association of immunity and tolerance to host H-2 determinants in irradiated F<sub>1</sub> hybrid mice reconstituted with bone marrow cells from one parental strain. *J. Exp. Med.* **142**:321.
  34. Stout, R. D., and L. A. Herzenberg. 1975. The Fc receptor on thymus-derived lymphocytes. I. Detection of a subpopulation of murine T lymphocytes bearing the Fc receptor. *J. Exp. Med.* **142**:1041.
  35. Stoecker, C. A., B. M. Rickard, and C. A. Abel. 1978. Effect of T- and B-lymphocyte mitogens on interactions between lymphocytes and macrophages. *Cell. Immunol.* **35**:362.
  36. McCombs, C., J. P. Michalski, and N. Talal. 1976. Cellular interactions in lymphocyte proliferation: effect of syngeneic and xenogeneic macrophages. *Cell. Immunol.* **23**:283.
  37. Katz, D. H., T. Hamaoka, and B. Benacerraf. 1973. Cell interactions between histoincompatible T and B lymphocytes. II. Failure of physiologic cooperative interactions between T and B lymphocytes from allogeneic donor strains in humoral response to hapten-protein conjugates. *J. Exp. Med.* **137**:1405.
  38. Pierce, C. W., J. A. Kapp, and B. Benacerraf. 1976. Regulation by the H-2 gene complex of macrophage-lymphoid cell interactions in secondary antibody responses in vitro. *J. Exp. Med.* **144**:371.
  39. Singer, A., K. S. Hathcock, and R. J. Hodes. 1979. Cellular and genetic control of antibody responses. V. Helper T-cell recognition of H-2 determinants on accessory cells but not B cells. *J. Exp. Med.* **149**:1208.
  40. Gengozian, N., C. C. Congdon, E. A. Allen, and R. E. Toya. 1971. Immune status of allogeneic radiation chimeras. *Transplant. Proc.* **3**:434.
  41. von Boehmer, H., L. Hudson, and J. Sprent. 1975. Collaboration of histoincompatible T and B lymphocytes using cells from tetraparental bone marrow chimeras. *J. Exp. Med.* **142**:989.

42. Erb, P. B., B. Miller, T. Matsunaga, and M. Feldman. 1979. Nature of T-cell macrophage interaction in helper-cell induction in vitro. II. Two stages of T-helper-cell differentiation analyzed in irradiation and allophenic chimeras. *J. Exp. Med.* **149**:686.
43. Hong, R., H. Schulte-Wissermann, E. Jarrett-Toth, S. D. Horowitz, and D. D. Manning. 1979. Transplantation of cultured thymic fragments. II. Results in nude mice. *J. Exp. Med.* **149**:398.
44. Katz, D. H., L. R. Katz, C. A. Bogowitz, and B. J. Skidmore. 1979. Adaptive differentiation of murine lymphocytes. II. The thymic microenvironment does not restrict the cooperative partner cell preference of helper T cells differentiating in  $F_1 \rightarrow F_1$  thymic chimeras. *J. Exp. Med.* **149**:1360.
45. Miller, J. F. A. P., M. A. Vadas, A. Whitelaw, and J. Gamble. 1976. Role of major histocompatibility complex gene products in delayed-type hypersensitivity. *Proc. Natl. Acad. Sci. U. S. A.* **73**:2486.
46. Asherson, G. L., B. Mayhew, and M. A. C. C. Perera. 1979. The production of contact sensitivity by the injection into footpads of recipients of the lymph node cells from mice 1 day after painting the skin with contact sensitizing agent: requirement for matching at the major histocompatibility complex between donor and recipient mice. *Immunology.* **32**:241.
47. Hunziken, N., and R. K. Winkelmann. 1978. Langerhans cells in contact dermatitis of the guinea pig. *Arch. Dermatol.* **114**:1309.
48. Shelley, W. B., and L. Juhlin. 1976. Langerhans cells form reticuloepithelial trap for external contact antigens. *Nature (Lond.)*. **261**:46.
49. Klareskog, L., V. M. Tjernlund, U. Forsum, and P. A. Peterson. 1977. Epidermal Langerhans cells express Ia antigens. *Nature (Lond.)*. **268**:248.
50. Claman, H. N., S. D. Miller, and J. W. Moorhead. 1977. Tolerance: two pathways of negative immunoregulation in contact sensitivity to DNFB. *Cold Spring Harbor Symp. Quant. Biol.* **16**:105.
51. Sy, M.-S., S. D. Miller, T. W. Moorhead, and H. N. Claman. 1979. Active suppression of 1-fluoro-2,4-dinitrobenzene-immune T cells. Requirement of an auxiliary T cell induced by antigen. *J. Exp. Med.* **149**:1197.
52. Conlon, P. J., J. W. Moorhead, and H. N. Claman. 1979. Efficient induction of immediate tolerance to contact sensitivity by hapten-modified spleen cells requires Ia<sup>+</sup> cells compatible with recipient. *Nature (Lond.)*. **278**:257.