

RESTRICTED ANTIBODY FORMATION TO SHEEP
ERYTHROCYTES OF ALLOGENEIC BONE MARROW
CHIMERAS HISTOINCOMPATIBLE AT THE
K END OF THE H-2 COMPLEX*

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Allogeneic bone marrow chimeras established from a combination of donor and recipient, which differ at the major histocompatibility barrier (H-2), show apparent immunodeficiency in primary plaque-forming cell (PFC) responses against T-dependent antigens such as sheep erythrocytes (SRBC) (1-3). Although several mechanisms have been proposed for the interpretation of the deficiency, e.g., the generation of suppressor T cells (4, 5), the lack of T cell clones that recognize their own H-2 products as self (6, 7), and the failure in T cell maturation (8), the exact nature of this deficiency is still poorly understood, and studies are far from complete.

This report presents evidence that a vigorous primary PFC response to SRBC occurs in allogeneic bone marrow chimeras when donor and recipient are compatible at the left one-half of the H-2 complex. By contrast, primary PFC response is very weak when incompatibility of donor and recipient is present on the left side of the major histocompatibility complex (MHC). Our findings support a definitive role for the thymic epithelial cells of the recipient mice in T cell differentiation; they are compatible with the view that donor T cells differentiating in the thymus of the recipient become able to recognize their partner cells through cell contact during development.

Materials and Methods

Mice. AKR/J (AKR, H-2^k), C56BL/6 (B6, H-2^b), C57BL/10 (B10, H-2^b), B10.BR (BR, H-2^k), B10.AKM (AKM, H-2^{m1}), B10.A (H-2^{a1}), and B10.A5R (5R, H-2^b) mice were obtained from The Jackson Laboratory, Bar Harbor, Maine. 8-12-wk-old female mice were used throughout the study.

Chimeras. Irradiation bone marrow chimeras were prepared by the method previously described (3, 9) in which bone marrow cells were treated with anti-Thy-1 serum alone (without complement). All the chimeras were maintained without further manipulation after hematopoietic reconstitution for 2.5-3.5 mo before analyses for chimerism and immunologic functions. Chimerism was tested by analyzing thymus cells for susceptibility to cytolysis by the specific anti-Thy-1.1 or Thy-1.2 antisera plus selected rabbit complement ([3, 9]; and K. Onoé, G. Fernandes, F.-W. Shen, and R. A. Good, unpublished data). None of the cells from the chimeras showed recipient type (Thy-1.1) specificity.

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TABLE I
Spleen Cell Responses to Mitogens in Chimeras

Group	Control*	Mitogen		
		Con A	PHA	LPS
[B10→AKR] (4)‡	888 ± 68§	37,905 ± 6,799	16,332 ± 3,465	30,323 ± 4,332
[BR→AKR] (5)	630 ± 147	15,509 ± 81	8,215 ± 2,801	28,400 ± 3,065
[B10.A→AKR] (4)	975 ± 294	32,110 ± 6,382	11,753 ± 3,326	42,024 ± 5,009
[5R→AKR] (4)	744 ± 164	27,379 ± 3,196	5,690 ± 354	30,755 ± 5,969
[AKM→AKR] (5)	530 ± 11	25,342 ± 4,675	5,291 ± 420	28,549 ± 2,864
[B6→B6] (2)	2,046 ± 40	21,726 ± 5,413	8,113 ± 3,423	34,566 ± 70
B10 (3)	1,166 ± 222	17,570 ± 3,464	12,289 ± 3,147	23,713 ± 662
AKR (3)	951 ± 360	24,240 ± 2,462	13,052 ± 3,018	25,318 ± 2,247

* The background counts in cultures without mitogen.

‡ Number of animals in each group.

§ Data are expressed as the average counts per minute of [³H]thymidine ± SE.

|| Age-matched nontreated mice.

Chimeras prepared by injecting C57BL/10 cells into irradiated AKR mice will be referred to as [B10→AKR]; AKR mice treated with B10.BR cells as [BR→AKR]; AKR mice treated with B10.AKM cells as [AKM→AKR]; AKR mice treated with B10.A cells as [B10.A→AKR]; AKR mice treated with B10.A(5R) cells as [5R→AKR], and B6 mice treated with irradiation plus syngeneic cells as [B6→B6].

PFC Assay to SRBC and 2,4-Dinitrophenyl (DNP)-Ficoll. The primary immune responses were quantified by the PFC assay of Jerne described previously (3, 9–11).

Mitogen Stimulation. Details of these assays have been described previously (3).

Results

Responses of Spleen Cells of Fully H-2-incompatible and Partially or Totally H-2-compatible Bone Marrow Chimeras to Mitogens. To evaluate the lymphocyte reconstitution, proliferative responses of the radiation chimeras induced by optimal concentration of concanavalin A (Con A), phytohemagglutinin (PHA), or lipopolysaccharide of *Escherichia coli* (LPS) were studied. A representative experiment comparing responses to these mitogens of spleen cells from the chimeras and unmanipulated B10 and AKR control mice is shown (Table I). It will be seen from Table I that although the proliferative responses to PHA were significantly lower for the [5R→AKR] and [AKM→AKR] chimeras than those for B10 or AKR control mice, they were comparable to those of [B6→B6] reconstituted by syngeneic marrow transplantation. It was noted that though in this particular experiment, considerable variation was observed in the proliferative responses of the individual chimeric mice, these responses were in the same general range as those of B10 or AKR control mice. These findings are interpreted as establishing that all the chimeras produced in this experiment using the combination of B10 H-2 recombinant mice as donor of bone marrow and AKR as recipient contain substantial populations of both T and B lymphocytes, which can respond by vigorous proliferation to stimulation with the mitogens. These results support the findings of our previous report in which fully allogeneic bone marrow chimeras [B6→AKR] showed a normal range of proliferative responses to Con A, PHA, and LPS as well as natural killer (NK) and antibody-dependent cell-mediated cytotoxicity (ADCC) activities (3).

Primary PFC Responses in In Vivo Stimulation with SRBC, a T-dependent Antigen, in H-2-incompatible and Partially or Totally H-2-compatible Chimeras. To evaluate capacity for T cell-dependent antibody production, the response to SRBC 4 d after primary antigenic stimulation was studied, using a plaque-forming assay. As shown in the previous

TABLE II
Primary Anti-SRBC and Anti-TNP-SRBC PFC Responses in Chimeras*

Group	H-2 region shared‡								PFC/spleen§		
	K	I					S	G	D	Anti-SRBC	Anti-TNP-SRBC
		A	B	J	E	C					
[B10→AKR]	b	b	b	b	b	b	b	b	9 ± 6	(6)	10,400 ± 2,800 (3)
[BR→AKR]	<u>k</u>	<u>k</u>	<u>k</u>	<u>k</u>	<u>k</u>	<u>k</u>	<u>k</u>	<u>k</u>	129,200 ± 11,800	(7)	15,400 ± 400 (4)
[B10.A→AKR]	<u>k</u>	<u>k</u>	<u>k</u>	<u>k</u>	<u>k</u>	<u>d</u>	<u>d</u>	<u>d</u>	49,900 ± 15,600	(6)	7,800 ± 1,100 (4)
[5R→AKR]	<u>b</u>	<u>b</u>	<u>b</u>	<u>k</u>	<u>k</u>	d	d	d	2,200 ± 900	(7)	13,400 ± 4,400 (4)
[AKM→AKR]	<u>k</u>	<u>k</u>	<u>k</u>	<u>k</u>	<u>k</u>	<u>k</u>	<u>k</u>	<u>q</u>	98,800 ± 9,900	(5)	10,400 ± 300 (3)
[B6→B6]	<u>b</u>	<u>b</u>	<u>b</u>	<u>b</u>	<u>b</u>	<u>b</u>	<u>b</u>	<u>b</u>	—		9,900 ± 100 (4)
B10									114,400 ± 10,000	(2)	15,000 ± 300 (4)
AKR									212,332 ± 35,000	(3)	28,400 ± 3,500 (5)

* 4 d after immunization with 0.2 ml of 10% SRBC for anti-SRBC PFC; 20 µg DNP-Ficoll in 0.2 ml PBS for anti-TNP-SRBC.

‡ Underlined regions are histocompatible between donor and recipient.

§ Data are expressed as the average PFC numbers ± SE.

|| See Table I, footnote ‡.

report (3), [B10→AKR] fully allogeneic chimeras were almost completely unresponsive to primary antigenic stimulation with SRBC (Table II). [5R→AKR] chimeras established by the combination of donor and recipient mice, which were histocompatible at only the I-J,E subregion of the H-2 complex, also showed negligible responses to SRBC. In contrast, [BR→AKR] fully H-2-compatible chimeras and [AKM→AKR] chimeras where donor and recipient differed from one another at only the H-2D locus showed numbers of PFC equal to or approaching those of the unmanipulated B10 controls. In [B10.A→AKR] chimeras where donor and recipient were compatible at the left one-half of H-2, primary PFC responses to SRBC were slightly lower than those in B10 control mice but substantially vigorous. From these results, it is suggested that in allogeneic bone marrow chimeras, histocompatibility at the K end of the H-2 between donor and recipient mice is required for the chimeras to show a primary antibody response to T-dependent antigen.

Primary PFC Responses to In Vivo Stimulation with DNP-Ficoll, a T-independent Antigen, in H-2-incompatible and Partially or Totally H-2-compatible Chimeras. Analyses to evaluate capacity to produce antibody to DNP-Ficoll, a T-independent antigen, gave quite different results. Regardless of whether donor and recipient mice were histocompatible, almost the same level of PFC responses to 2,4,6-trinitrophenyl (TNP)-SRBC was seen in chimeric mice (Table II). The number of PFC of the chimeras was at a level similar to that of B10 donor mice. This observation too fits well with those of our prior report (9) in which the degree of the response to DNP-Ficoll was linked to the donor type of bone marrow cells and not to the responses of the recipient strain. The quantity of such responses is most likely inheritable, but controlled by non-H-2 and non-Ig heavy chain genes (11). The results presented in Table II also are consonant with our previous findings (9, 11) because allogeneic bone marrow chimeras prepared with bone marrow cells of mice of B10 H-2-congenic lines uniformly showed low responsiveness to DNP-Ficoll.

Discussion

In previous reports, we described a reproducible method for preparing long-lived allogeneic chimeras where donor and recipient mice differed from one another at the MHC barrier, H-2 ([3, 9]; and K. Onoé, G. Fernandes, F.-W. Shen, and R. A. Good,

unpublished observations). With this method, long-lived chimeras were established by reconstituting lethally irradiated recipient mice with allogeneic bone marrow cells that had been pretreated with anti-Thy-1 serum without complement. Although these fully allogeneic bone marrow chimeras had substantial populations of both T and B lymphocytes and both NK and ADCC cells, they showed gross deficiency in the primary PFC response to SRBC, a T-dependent antigen (3).

In the present paper, in putting this new method to practical use, we have prepared a number of irradiated, bone marrow-reconstituted chimeras constructed from various combinations of marrow cells from B10 H-2 recombinant strains and AKR recipient mice. In these chimeras, cellular components involved in immune responses were totally or partially H-2 compatible regardless of other genetic contributions, because B10 recombinant strains of mice used in these experiments differed from one another only within the H-2 complex. In these chimeras, virtually all the thymus cells were shown to be Thy-1.2⁺ and, hence, to be derived from the donor of B10 recombinant mice. Most of these chimeras, regardless of the source of marrow cells, showed the normal ranges of proliferative responses to both T and B cell mitogens (Table I), which confirms and extends our previous observations (3). Support for the prior findings was also derived from the experiment that showed normal responses to TNP-SRBC (Table III) in all of the chimeras, because a B cell subset could mature into antibody-producing cells (9). Thus, these mice had well-developed populations of T and B cells that showed little or no evidence of being immunodeficient. We then tried to determine which combinations showed primary PFC responses to SRBC.

As we had shown in the previous report (3), fully allogeneic chimeras [B10→AKR] were almost completely unresponsive to SRBC, and [5R→AKR] I-J,E-region-compatible chimeras also showed negligible responses to SRBC (Table II). However, [BR→AKR] fully H-2-compatible chimeras and [AKM→AKR] chimeras where donor and recipient differed at only H-2D, showed the same numbers of PFC as B10 control mice. In [B10.A→AKR] chimeras, where donor and recipient were matched at the K-I-E region of the H-2, slightly lower but still vigorous responses were observed that were comparable to those seen in the B10 control. These results demonstrate that the primary PFC response to SRBC is dependent upon cellular interactions generated when the K end of the H-2 complex is compatible between donor bone marrow cells and other cells of the recipient in our system of allogeneic bone marrow transplantation. These observations also seem to be concordant with the previous findings by Sprent (12) and Sado and Kamisaku (13) in which it was found that the restricted helper function of T cells from semiallogeneic chimeras or congenic resistant strains of mice is controlled by the K end of the H-2 complex.

The activation of T cells by antigen is also controlled by gene products of the MHC. There is a requirement for H-2K or H-2D matching between cytotoxic T cells and their targets (14); for H-2I matching between helper T cells and B cells (15); and for H-2I matching between T cells and macrophages involved in delayed type-hypersensitivity (16). These restrictions, however, may not merely depend on the H-2 haplotype of the responding T cells, but rather depend upon the H-2 determinants on the thymic epithelial cells which the T cells encounter in the thymus during early stages of differentiation (6, 7, 17). According to this idea, our present finding supports the definitive role of the recipient microenvironment, e.g., thymic epithelium, on the generation of the primary antibody responses to SRBC. Because almost all of the lymphocytes of the chimeras are of donor origin, the cells, e.g., macrophages, T and

B cells, bear the same H-2 products on their surface. There were striking differences, however, in the primary PFC responses to SRBC among those chimeras that relate to the H-2 matching of the donor marrow cells and the cells of the recipient mice. Only when T cells encounter their own MHC products, the K end of the H-2, in the thymus, do they develop cells which, with macrophages, can recognize antigens and collaborate to produce antibody with B cells and macrophages, both bearing the same H-2 products on their surface as the thymic epithelium. The possibility, however, that other components of the recipient microenvironment may exert an influence on cooperative capabilities of helper T cells, as proposed by Katz et al. (18), can not be ruled out. The latter investigators demonstrated that helper T cells with unrestricted cooperating phenotypes may be induced in F_1 →parent chimeric populations when adoptively primed in irradiated F_1 recipients.

The absolute lack of antibody formation to SRBC in H-2-incompatible bone marrow chimeras is restricted to the primary response. We have shown that vigorous PFC response to SRBC occurs even in the fully allogeneic bone marrow chimeras after a second stimulation by the antigen (3). The exact mechanism of this essential difference between primary and secondary antibody responses to T-dependent antigen now requires critical analysis.

Summary

Employing a new method for allogeneic bone marrow transplantation, irradiation chimeras constructed from various combinations of marrow cells from B10 H-2 recombinant mice and AKR recipients were prepared. Though these chimeras had well-developed populations of T and B cells, they showed strikingly different patterns of responses in the primary antibody formation to sheep erythrocytes (SRBC), a T-dependent antigen. These are (a) AKR mice treated with C57BL/10 cells, [B10→AKR] fully H-2-incompatible, and AKR mice treated with B10.A (5R) cells, [5R→AKR] I-J,E-compatible chimeras that were almost completely unresponsive to SRBC; (b) AKR mice treated with B10.BR cells, [BR→AKR] fully H-2-compatible, and AKR mice treated with B10 AKM cells, [AKM→AKR] chimeras where donor and recipient differed only at H-2D, showed the same number of plaque-forming cells (PFC) as B10 control mice; (c) AKR mice treated with B10.A cells, [B10.A→AKR] chimeras, where donor and recipient were matched at H-2K-I-E region, showed about one-half the number of PFC as the control mice. From these results we conclude that in allogeneic bone marrow chimeras primary antibody response to T-dependent antigen, such as SRBC, is generated when at least the K end of the H-2 complex is compatible between donor and recipient.

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