

SELF RECOGNITION IN ALLOGENEIC RADIATION BONE MARROW CHIMERAS

A Radiation-resistant Host Element Dictates the Self Specificity and Immune Response Gene Phenotype of T-Helper Cells

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Products of genes encoded within the major histocompatibility complex (MHC)¹ perform a critical role in the recognition by T cells of conventional (non-MHC) antigens in that T cells recognize conventional antigens presented in the context of specific self MHC determinants (1-4). Studies with T cells from semiallogeneic ($A \rightarrow A \times B$ and $A \times B \rightarrow A$) radiation bone marrow chimeras have suggested that the selection of specific MHC determinants to serve as self structures for the recognition of conventional antigens is not determined by the genotype of the T cells, but rather is dictated by the MHC phenotype of the environment in which T cell precursors differentiate (5-11). However, two different sets of experimental results have raised serious doubts concerning the validity of the concept that self recognition is a consequence of the MHC determinants expressed in the differentiation environment in which T cell precursors mature.

First, most experiments utilizing T cells from fully allogeneic ($A \rightarrow B$) radiation bone marrow chimeras have failed to fulfill the prediction that $A \rightarrow B$ T cells would be restricted to the self recognition of only B MHC determinants (7, 8, 12, 13). Indeed, some studies with fully allogeneic chimeras have failed to detect the existence of any competent T cell function by cells of donor bone marrow origin (7, 8, 12), whereas other studies in which competent T cell function was observed found that the receptor repertoire of these T cells was not restricted to the self recognition of host MHC determinants (13). Second, a number of acute-depletion-type experiments have failed to fulfill the prediction that strain A T cell populations that had been acutely depleted of anti-B alloreactivity would be restricted to the self recognition of only syngeneic A MHC determinants (14-17). In these experiments, strain A T cells that had matured in a normal strain A environment and had been acutely depleted of alloreactive anti-B specific cells were able to recognize both A and B self MHC determinants. Because

¹ *Abbreviations used in this paper:* B + Accessory cells, spleen cells from which T cells were removed by pretreatment with either a T cell-specific cytotoxic rabbit anti-mouse brain reagent or with a monoclonal anti-Thy-1.2 reagent plus complement; C, complement; CML, cell-mediated lympholysis, CTL, cytotoxic T lymphocytes; KLH, keyhole limpet hemocyanin; MHC, major histocompatibility complex; NNA, nylon nonadherent; PBL, peripheral blood lymphocytes; PFC, plaque-forming cells; RAMB, rabbit anti-mouse brain serum; (T,G)-A-L; poly-L-(Tyr,Glu)-poly-D,L-Ala--poly-L-Lys; TNP, trinitrophenyl; SAC, spleen-adherent cells, SRBC, sheep erythrocytes.

of these apparently contradictory results, it was important to reevaluate the concept that T cell self-recognition is determined by the MHC phenotype of the environment in which T cell precursors differentiate.

In this study, the tolerance, competence, and self recognition specificities of T cells from semiallogeneic ($A \rightarrow A \times B$ and $A \times B \rightarrow A$) and fully allogeneic ($A \rightarrow B$) chimeras were evaluated. An additional type of chimera, referred to as a double donor chimera ($A + B \rightarrow A$), was constructed so that the self recognition specificities could be evaluated for homozygous T cells that matured in a syngeneic A environment while being tolerized to allogeneic B MHC determinants. The T cell self recognition repertoire expressed in these experimental animals, was assessed utilizing a primary in vitro T-helper cell-dependent plaque-forming cell (PFC) response as the experimental assay so that two requirements could be fulfilled that are critical for the accurate evaluation of T cell self recognition in complicated chimeric animals: First, all of the interacting cell populations were derived from unprimed animals to avoid potential distortions in the T cell repertoire of chimeric mice resulting from the selective expansion of those T cells triggered by the immunizing antigen in association with the antigen-presenting accessory cells resident in the chimeras. Second, antigen-presenting accessory cells were always clearly identified and added separately into the cultures, avoiding uncertainties as to the MHC phenotype of the antigen-presenting accessory cells functioning in these responses. The results of experiments so constructed clearly demonstrate that the MHC determinants which T-helper cells recognize as self structures are those expressed on a radiation-resistant element in the environment in which T-helper cells differentiate.

Materials and Methods

Animals. C57BL/10Sn (B10), B10.A, and (B10 \times B10.A) F_1 mice were obtained from The Jackson Laboratory, Bar Harbor, Maine. Males 2-5 mo old were used in all experiments.

Chimeras. Chimeras are designated as bone marrow donor \rightarrow irradiated recipient. Recipient mice for $A \rightarrow A \times B$, $A \times B \rightarrow A$, and $A \rightarrow B$ chimeras were irradiated with 950 rad (x ray) and reconstituted 2-6 h later with 15×10^6 bone marrow cells that had been pretreated with rabbit anti-mouse brain serum (RAMB) and complement (C) as previously described (11). Mice for $A + B \rightarrow A$ chimeras were similarly constructed except that they were reconstituted with 10×10^6 bone marrow cells of strain A and 20×10^6 bone marrow cells of strain B. Spleen cells were obtained from each chimera no earlier than 2 mo postirradiation and were individually typed by indirect immunofluorescence using H-2-specific reagents as previously described (11). By such testing, spleen cells from each chimera (except the double donor chimeras) were of donor origin without detectable (<5%) cells of host origin. In the case of double donor chimeras, their peripheral blood lymphocytes (PBL) were first typed by indirect immunofluorescence to ascertain that each chimera was balanced. At the time of the experiment, the spleen cells from $A + B \rightarrow A$ chimeras were typed to further confirm that each double donor chimera contained cells of each donor haplotype. No discrepancy between the typing of PBL and spleen cells were observed. All chimeras were housed in a limited-access facility, with long-term survival >60% for every type of chimera.

Antigens. Keyhole limpet hemocyanin (KLH; lot 530195; Calbiochem-Behring Corp., American Hoechst Corp., San Diego, Calif.) and poly-L-(Tyr,Glu)-poly-D,L-Ala-poly-L-Lys [(T,G)-A-L] (lot MC-8; Miles-Yeda Research Laboratories, Rehovot, Israel) were conjugated with 2,4,6-trinitrobenzene sulfonate (Pierce Chemical Co., Rockford, Ill.) as previously described (18). The concentration of each antigen used in culture was the optimal concentration for that antigen and was 5-10 $\mu\text{g}/\text{ml}$ final concentration trinitrophenyl (TNP)-KLH and 0.8 $\mu\text{g}/\text{ml}$ TNP-(T,G)-A-L.

Preparation of Cell Subpopulations. T cells were prepared by passage of spleen cells over nylon

fiber columns and collection of the nylon-nonadherent (NNA) eluate (18). Cells, designated B + accessory cells, were prepared by depleting spleen cells of T cells by pretreatment with either a T cell-specific cytotoxic RAMB reagent (18) or with a monoclonal anti-Thy-1.2 reagent + C, which was the generous gift of Dr. P. Lake, University College, London, England. Spleen cells were depleted of adherent accessory cells by passage over G-10 Sephadex columns. This procedure markedly reduces the percentage of latex-ingesting cells although not significantly altering the percentage of T cells or B cells (19). Spleen adherent cells (SAC) were utilized as accessory cell populations and were prepared by 2-h adherence to glass followed by treatment with RAMB + C and irradiation with 1,000 rad (19).

Anti-H-2K^k + C Treatment of Cells. Monoclonal anti-H-2K^k reagent was a culture supernate of the hybridoma 11-4.1, described by Oi et al. (20) and obtained from the Cell Distribution Center of the Salk Institute (La Jolla, Calif.). 5×10^6 cells/ml were treated with a 1:4 dilution of this reagent for 30 min at 37°C followed by treatment with a 1:6 dilution of rabbit C for an additional 30 min at 37°C.

Culture Conditions for the In Vitro Generation of PFC. All cultures were performed in a volume of 200 μ l per flat-bottomed well of microtiter plates and were incubated for 4 d in a 5% CO₂-humidified air atmosphere as previously described (18). The cell number in each culture was 5×10^6 cells or as otherwise stated. Sheep erythrocytes (SRBC) were conjugated with TNP (TNP-SRBC) and direct IgM PFC to TNP-SRBC were assayed by the slide modification of the Jerne hemolytic plaque technique (18). All points shown in each experiment represent the geometric mean responses of at least three replicate cultures for TNP-KLH or replicate culture groups (each culture group consisted of a pool of two individual cultures) for TNP-(T,G)-A-L.

Culture Conditions for the Generation of Cytotoxic T Lymphocytes (CTL). All cultures consisted of 2×10^6 – 4×10^6 responding spleen cells and 1×10^6 2,000-rad irradiated stimulator spleen cells in a volume of 2 ml minimum essential medium-fetal calf serum incubated for 5 d in a 5% CO₂-humidified air atmosphere. Cells were harvested and assayed for their ability to lyse ⁵¹Cr-labeled concanavalin A blasts as target cells in a 4-h chromium-release assay (21).

Results

Assessment of Alloreactivity and Tolerance in Semiallogeneic ($A \rightarrow A \times B$ and $A \times B \rightarrow A$), Fully Allogeneic ($A \rightarrow B$), and Double Donor ($A + B \rightarrow A$) Radiation Bone Marrow Chimeras. The alloreactivity of chimeric spleen cells was assessed by their ability to generate cell-mediated lympholytic (CML) responses against B10 (H-2^b), B10.A (H-2^a), and third-party B10.D2 (H-2^d) MHC determinants. As can be seen in Fig. 1,

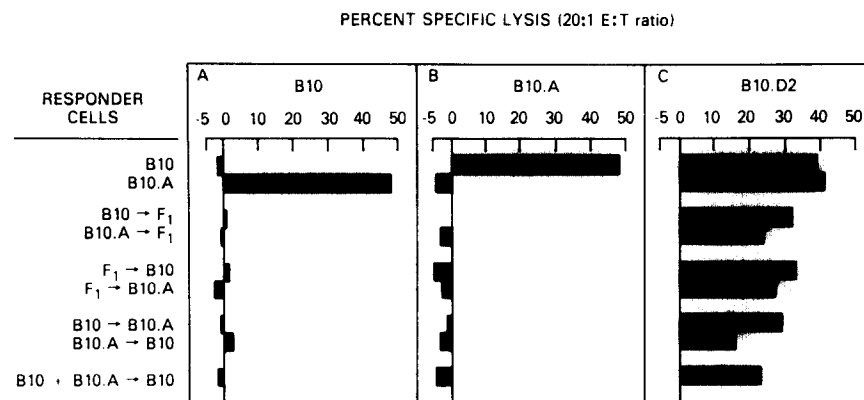


FIG. 1. Assessment by CML of alloreactivity in allogeneic chimeras. Specific CTL activity generated by various responder cell populations was assayed on concanavalin A-induced spleen cell blasts as target cells, which were syngeneic to the stimulator cells. The percent specific lysis was determined at a 20:1 effector:target (E:T) ratio in a 4-h ⁵¹Cr-release assay. Spontaneous release by each target cell in this experiment ranged between 25 and 27%.

radiation bone marrow chimeras of all combinations contained T cells that were competent to recognize and respond to third party H-2^d alloantigens. In contrast, none of the chimeric cell populations reacted significantly against either H-2^a or H-2^b determinants, indicating that these populations were tolerant to those MHC determinants. Similar results were obtained with chimeric spleen cells when assayed by mixed lymphocyte reactions (data not shown). Thus, the T cell populations present in these chimeras were tolerant to their own MHC determinants, to the MHC determinants expressed by the chimeric host, and to the MHC determinants expressed on other lymphoid cells present in their differentiation environment.

Intact Spleen Cell Populations from Fully Allogeneic Chimeras Are Not Competent to Generate In Vitro Primary PFC Responses to TNP-KLH. As a first step in the analysis of the self specificity of chimeric T-helper cells, the ability of unfractionated spleen cells from chimeras to generate T-helper cell-dependent primary in vitro anti-hapten PFC responses to soluble TNP-KLH was determined (Table I). Spleen cells from A → A × B, A × B → A, and A + B → A chimeras all generated significant primary PFC responses to TNP-KLH, demonstrating the presence in these mice of functionally competent cell populations. In contrast, intact spleen cell populations from fully allogeneic chimeras (B10 → B10.A and B10.A → B10) failed to generate any response to TNP-KLH (Table I). Such unresponsiveness by spleen cells from fully allogeneic chimeras suggested that: (a) one or more of the cell populations in allogeneic chimeras had not matured into functional competence; or (b) each individual cell population was functionally competent but that at least one cell population (e.g., T-helper cells)

TABLE I
Intact Spleen Cells from Fully Allogeneic Chimeras Are Incapable of Generating In Vitro Responses to TNP-KLH

Responder spleen cells	PFC/culture*	
	TNP-KLH	No antigen
A. B10	184(1.13)	0
B10.A	118(1.55)	0
(B10 × B10.A)F ₁	175(1.23)	2(1.82)
B10 → (B10 × B10.A)F ₁	102(1.30)	0
B10.A → (B10 × B10.A)F ₁	126(1.12)	1(1.25)
(B10 × B10.A)F ₁ → B10	252(1.27)	5(1.09)
(B10 × B10.A)F ₁ → B10.A	143(1.11)	0
B10 → B10.A	1(1.25)	1(1.0)
B10.A → B10	1(1.25)	1(1.25)
B10 + B10.A → B10	90(1.07)	1(1.81)
B10 + B10.A → B10.A	185(1.16)	5(2.36)
B. B10 → B10.A	9(1.75)	0
B10.A → B10	8(1.31)	2(1.65)
B10 → B10.A + B10.A → B10	327(1.05)	0

* Geometric mean of triplicate cultures, SE is in parentheses.

was restricted to interacting with cells expressing host and not donor MHC determinants; or (c) responses were nonspecifically but actively suppressed.

To distinguish among these possibilities, a cell-mixing experiment was performed. Unfractionated spleen cells from an unresponsive B10 \rightarrow B10.A chimera were cocultured with an equal number of unfractionated spleen cells from a reciprocal and equally unresponsive B10.A \rightarrow B10 chimera. The mixed population of chimeric spleen cells should remain unresponsive to TNP-KLH if the immune cell populations in the spleens of these mice were either functionally incompetent or nonspecifically suppressed. However, if the immune cell populations in the spleens of these mice were competent but host restricted in their cellular interactions, then the coculture of B10 \rightarrow B10.A and B10.A \rightarrow B10 spleen cells should be responsive to TNP-KLH. As can be seen in Table IB, cocultures of B10 \rightarrow B10.A and B10.A \rightarrow B10 spleen cells were competent to generate responses to TNP-KLH. Thus, these results demonstrate that each of the cell populations required for the generation of primary PFC responses to TNP-KLH did mature into functional competence in an allogeneic MHC environment and suggest that the lymphocytes from fully allogeneic chimeras are restricted to the self recognition of host MHC determinants.

Restricted Self Recognition by Chimeric Lymphocytes of MHC Determinants Expressed by Accessory Cells. The possibility that lymphocytes from fully allogeneic chimeras were competent but host restricted in their self recognition repertoire was assessed by examining the ability of chimeric lymphocytes to recognize and cooperate with accessory cells expressing either donor or host MHC determinants. Spleen cell populations from A \rightarrow A \times B, A \times B \rightarrow A, and A \rightarrow B chimeras were depleted of adherent accessory cells by passage over G-10 Sephadex, a procedure which also abrogated the ability of the remaining (T + B) cells to respond to soluble TNP-KLH (Fig. 2). Consistent with previously reported results (11), lymphocytes from A \rightarrow A \times B chimeras were capable of cooperating with SAC from either parent A or B (Fig. 2A and B), whereas lymphocytes from A \times B \rightarrow A lymphocytes were restricted to cooperating only with SAC expressing the MHC determinants of parent A (Fig. 2C and D). More importantly, the lymphocytes from fully allogeneic A \rightarrow B chimeras were similarly competent and restricted to cooperating with accessory cells expressing only host-type MHC determinants (Fig. 2E and F).

Even though no residual host cells were ever detected in the spleens of these chimeras by indirect immunofluorescence, it was important to consider the possibility that the functionally competent and host MHC-specific lymphocytes present in A \rightarrow B chimeras might be of host B origin. Consequently, an experiment was performed in which the spleen cell populations from B10 \rightarrow B10.A chimeras were pretreated with monoclonal anti-H-2K^k + C to eliminate any residual host B10.A cells (Table II). As before, B10 \rightarrow B10.A spleen cells did not themselves respond to TNP-KLH; however, upon the addition of B10.A (but not B10) SAC, the chimeric lymphocytes were able to generate responses to TNP-KLH. Treatment of this B10 \rightarrow B10.A spleen cell population with anti-H-2K^k + C had very little effect on responsiveness (Table II). The minimal enhancement of responses observed after anti-H-2K^k + C treatment was consistently observed with treatment of all spleen cell populations with C alone. It should be noted that even though treatment with anti-H-2K^k + C had little effect on B10 \rightarrow B10.A spleen cells, such treatment lysed >98% of B10.A spleen cells (data not shown). Thus, this experiment demonstrates that the lymphocytes from A \rightarrow B

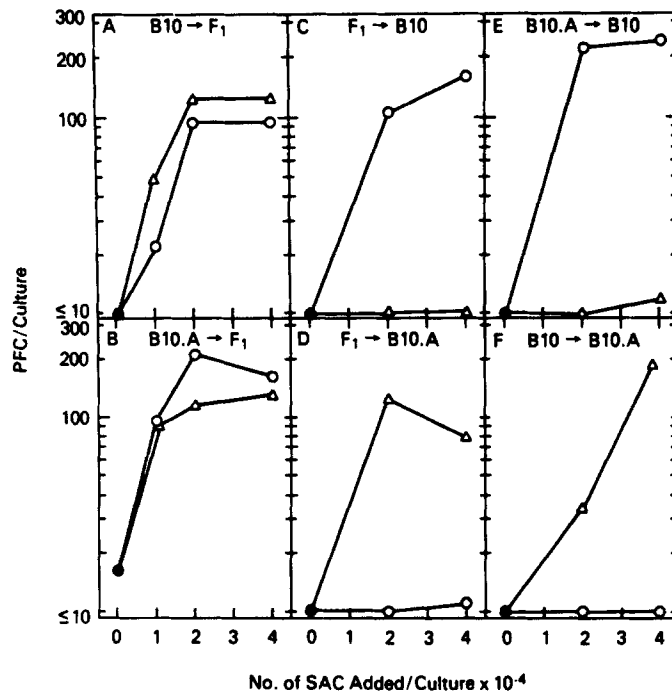


FIG. 2. Ability of lymphocytes from semiallogeneic and fully allogeneic chimeras to collaborate with accessory cells expressing host, but not donor, MHC determinants. Graded numbers of SAC from B10 (O) or B10.A (Δ) spleens were added to cultures containing TNP-KLH and 5×10^6 G-10-passed spleen cells from various chimeras. <5 PFC/culture were obtained in the absence of antigen.

allogeneic chimeras which function and express host restricted self recognition are not of host origin.

To determine directly whether T-helper cells were at least one of the lymphocyte populations in A → B allogeneic chimeras which were restricted in their MHC-specific self recognition repertoire, NNA T cells from the spleens of fully allogeneic B10 → B10.A and B10.A → B10 chimeras were assayed for their ability to cooperate with either B10 or B10.A B + Accessory cells. As can be seen in Fig. 3, B10 → B10.A T cells only cooperated with B10.A B + Accessory cells, whereas B10.A → B10 T cells only cooperated with B10 B + Accessory cells.

Three conclusions can be drawn from this series of experiments: First, it can be concluded that lymphocytes from fully allogeneic A → B chimeras are competent but restricted to interacting with accessory cells which express host, not donor, MHC determinants. The failure of unfractionated spleen cells from fully allogeneic chimeras to respond to TNP-KLH thus results from the absence in these chimeras of accessory cells expressing host MHC determinants. Second, it can be concluded that the only MHC-restricted cell interactions required for the generation in vitro of these primary PFC responses occurs between lymphocytes and accessory cells, because otherwise the addition of host strain B accessory cells to cultures of spleen cells from A → B fully allogeneic chimeras would not have been sufficient to reverse the unresponsiveness of these spleen cells. Third, it can be concluded that the MHC determinants that mature

TABLE II
The Functional Lymphocyte Populations in Fully Allogeneic Chimeras Are Not of Host Origin

Responding spleen cells	Treatment	Strain of added SAC	Number of Added SAC $\times 10^4$	PFC/culture*		
				TNP-KLH	No antigen	
B10 \rightarrow B10.A	—	—	—	2(2.03)	2(2.00)	
			B10	4	2(1.60)	0
				2	4(2.23)	
		1		5(1.22)		
		B10.A	4	107(1.35)	0	
			2	125(1.04)		
1	5(1.18)					
B10 \rightarrow B10.A	anti-H-2K ^k + C	—	—	2(1.22)	0	
			B10	4	3(1.50)	0
				2	2(2.22)	
		1		8(1.06)		
		B10.A	4	163(1.34)	7(1.54)	
			2	275(1.13)		
			1	20(1.71)		

* Geometric mean of triplicate cultures, SE is in parentheses.

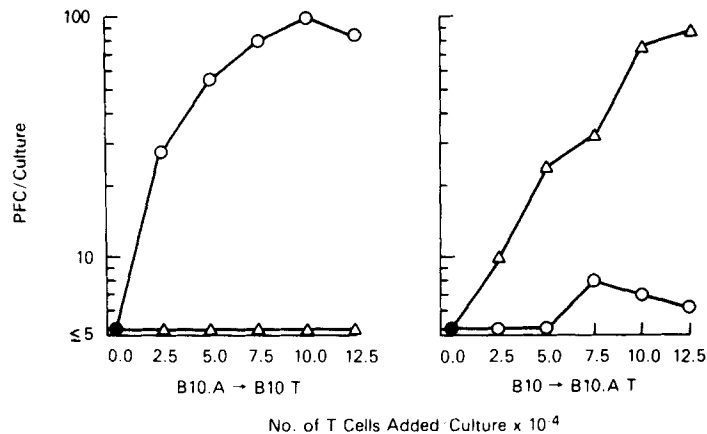


FIG. 3. Ability of T-helper cells from fully allogeneic chimeras to cooperate with B + Accessory cells expressing host, but not donor, MHC determinants. Graded numbers of T cells from either B10.A \rightarrow B10 or B10 \rightarrow B10.A chimeras were added to cultures containing TNP-KLH and 4×10^5 B + Accessory cells from either B10 (O) or B10.A (Δ) spleens. <5 PFC/culture were obtained in the absence of either B cells or antigen.

T-helper cells recognize as self structures are those that were expressed by the host environment in which their precursors matured.

Self Recognition by Lymphocytes from Double Donor (A + B \rightarrow A) Chimeras. Although the experiments presented thus far demonstrate that the MHC determinants recognized as self structures by chimeric T cells are those of the chimeric host, the possibility

must be considered that self recognition of allogeneic host MHC determinants might result simply from the tolerization of precursor T cells to these determinants and not from the influence of any specific host element, such as the thymus. It was to directly examine such a possibility that double donor A + B → A chimeras were constructed. The behavior of strain B lymphocytes from A + B → A double donor chimeras would not be expected to be particularly informative because these cells should be similar to the strain B lymphocytes from B → A allogeneic chimeras already examined. In contrast, the behavior of strain A lymphocytes from A + B → A double donor chimeras should be uniquely informative because these lymphocytes will have differentiated in a syngeneic A host environment while simultaneously being tolerized to allogeneic B MHC determinants.

The lymphocytes from all double donor chimeras were typed to ascertain that each animal had been repopulated by lymphocytes of both donor types. This was critical because the mice that had not been repopulated with strain B lymphocytes were not tolerant to B MHC determinants. Indeed, 86% of A + B → A chimeras which were constructed with 10^7 strain A and 2×10^7 strain B bone marrow cells contained

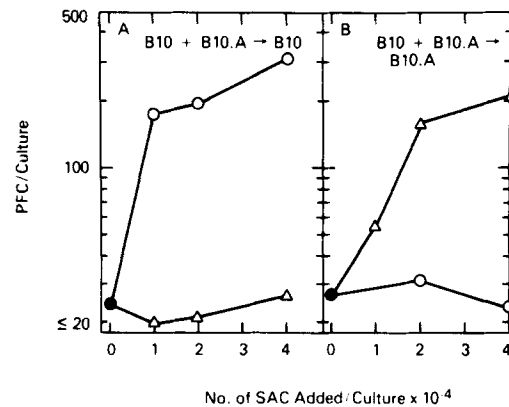


FIG. 4. Restricted recognition of lymphocytes from double donor chimeras of accessory cells. Graded numbers of SAC from B10 (○) or B10.A (△) were added to cultures containing TNP-KLH and 5×10^6 Sephadex G-10-passed spleen cells from B10 + B10.A → B10 chimeras (A) or B10 + B10.A → B10.A chimeras (B). <5 PFC/culture were obtained in the absence of antigen.

TABLE III

Strain A Lymphocytes from A + B → A Double Donor Chimeras Recognize as Self-Structures the MHC Determinants of Strain A but not Strain B

G-10-passed lymphocytes (5×10^5 /culture)	Lymphocyte treatment	Strain of added SAC (4×10^4 /cul- ture)	PFC/culture*	
			TNP-KLH	No anti- gen
B10 + B10.A → B10	—	—	14(1.62)	0
		B10	281(1.21)	0
		B10.A	56(1.28)	0
B10 + B10.A → B10	anti-H-2K ^k + C	—	21(1.22)	0
		B10	772(1.04)	0
		B10.A	64(1.01)	0

* Geometric mean of triplicate cultures, SE is in parentheses.

significant numbers of lymphocytes from both strain B ($36 \pm 2\%$) and strain A and were tolerant to both A and B MHC determinants (Fig. 1). As previously demonstrated, unfractionated spleen cells from $B10 + B10.A \rightarrow B10$ and $B10 + B10.A \rightarrow B10.A$ chimeras were competent to respond to TNP-KLH (Table IA). Removal of adherent accessory cells from such spleens by G-10 Sephadex passage markedly diminished their ability to respond to TNP-KLH (Fig. 4). The responses of lymphocytes from $B10 + B10.A \rightarrow B10$ chimeras were only reconstituted by the addition to culture of B10 SAC but not B10.A SAC, whereas the responses of lymphocytes from $B10 + B10.A \rightarrow B10.A$ chimeras were only reconstituted by the addition to culture of B10.A SAC but not B10 SAC (Fig. 4). These results suggest that even though the lymphocytes from double donor $A + B \rightarrow A$ chimeras were tolerant to both A and B MHC determinants, they were restricted to the self recognition of host A MHC determinants. To next assess the self recognition specificity of the strain A lymphocytes in these $A + B \rightarrow A$ double donor chimeras, B10 lymphocytes were isolated from $B10 + B10.A \rightarrow B10$ chimeras by eliminating the B10.A lymphocytes from the spleens of these mice by treatment with anti-H-2K^k + C. As can be seen in Table III, the marked preference of unseparated $B10 + B10.A \rightarrow B10$ lymphocytes for cooperation with B10 SAC was also expressed by the B10 lymphocytes isolated from these same chimeras.

Because strain A lymphocytes from $A + B \rightarrow A$ double donor chimeras were tolerized to allogeneic strain B MHC determinants but only recognized syngeneic host MHC determinants as self recognition structures, it can be concluded that the expression of a receptor repertoire specific for the self recognition of allogeneic MHC determinants is not merely a consequence of the tolerization of precursor T cells to

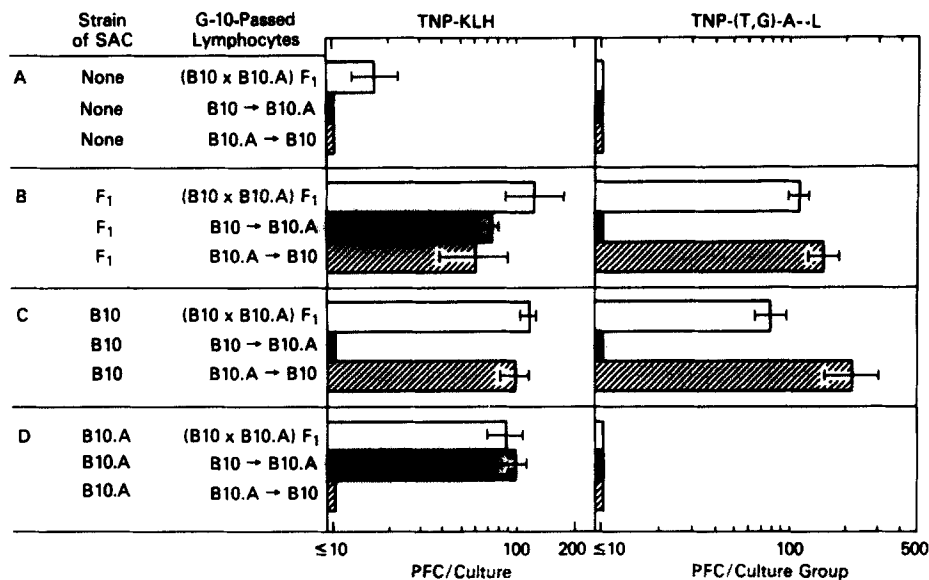


FIG. 5. Influence of the differentiation environment on the Ir gene phenotype expressed by lymphocytes. 2×10^4 SAC from (B10 x B10.A)F₁ (B), B10 (C), or B10.A (D) were added to cultures containing either TNP-KLH or TNP-(T,G)-A-L and 5×10^5 Sephadex G-10-passed spleen cells from (B10 x B10.A)F₁, B10 → B10.A, or B10.A → B10. < 5 PFC/culture were obtained in the absence of antigen.

allogeneic determinants. Rather, these results suggest that self recognition is specifically dictated by radiation-resistant host elements in the differentiation environment.

Complete Reversal of the Immune Response (Ir) Phenotype of Lymphocytes from Fully Allogeneic Chimeras. Because the MHC determinants that are utilized as self recognition structures by T-helper cells for responses to conventional antigens are determined by a radiation-resistant element in the T cell differentiation environment, it was of interest to determine if the ability of T-helper cells to respond to conventional antigens whose responses are regulated by MHC-linked Ir genes might similarly be determined by the T cell differentiation environment. Because the in vitro primary PFC responses to TNP-(T,G)-A--L are regulated by autosomal dominant H-2-linked Ir genes such that H-2^b mice are responders whereas H-2^a mice are nonresponders (19), it was possible to assess the Ir phenotype for responsiveness to TNP-(T,G)-A--L of B10 → B10.A and B10.A → B10 allogeneic chimeras by examining their in vitro PFC responses to this antigen.

Spleen cell populations from normal (B10 × B10.A)_{F1} and chimeric B10 → B10.A and B10.A → B10 mice were depleted of adherent accessory cells by G-10 Sephadex passage and reconstituted with (B10 × B10.A)_{F1} SAC (Fig. 5 A and B). In the presence of F₁ SAC all three lymphocyte populations were able to generate significant responses to TNP-KLH (Fig. 5 B). In contrast, this was not the case for the Ir gene controlled responses to TNP-(T,G)-A--L. Instead, whereas both normal F₁ lymphocytes and H-2^a (nonresponder genotype) lymphocytes from B10.A → B10 chimeras responded to TNP-(T,G)-A--L, H-2^b (responder genotype) lymphocytes from B10 → B10.A chimeras did not respond (Fig. 5). Because the B10 → B10.A lymphocytes were capable of cooperating with F₁ SAC for responses to TNP-KLH, the failure of these lymphocytes to cooperate with F₁ SAC for responses to TNP-(T,G)-A--L could not be a result of any generalized MHC barriers to their interaction. Further, because the F₁ SAC functioned competently to present TNP-(T,G)-A--L to the other two lymphocyte populations, the failure of B10 → B10.A lymphocytes to respond to TNP-(T,G)-A--L could not be attributed to an intrinsic failure of F₁ SAC to function in responses to TNP-(T,G)-A--L. Rather, the failure of B10 → B10.A lymphocytes to respond to TNP-(T,G)-A--L presented by competent F₁ SAC indicated that these lymphocytes were unresponsive to TNP-(T,G)-A--L even though they possessed the responder (H-2^b) genotype. Conversely, the ability of B10.A → B10 lymphocytes to respond to TNP-(T,G)-A--L presented by F₁ SAC indicated that these lymphocytes were responsive to TNP-(T,G)-A--L even though they possessed the nonresponder (H-2^a) genotype. In this same experiment, the ability of normal F₁ and chimeric lymphocytes to cooperate with parental responder and nonresponder SAC was also determined. As can be seen, responsive lymphocytes (normal F₁ and B10.A → B10) only generated responses to TNP-(T,G)-A--L in the presence of responder B10 but not nonresponder B10.A SAC (Fig. 5 C and D).

This experiment demonstrates for that class of Ir genes that regulates the responsiveness of H-2^a and H-2^b mice to TNP-(T,G)-A--L Ir gene function is expressed at both the accessory cell level and the lymphocyte level, and that the ability of lymphocytes to function in these responses is a consequence not of their genotype but of the Ir phenotype of the environment in which they differentiate. The striking parallel between the ability of lymphocytes to respond to TNP-(T,G)-A--L and their ability to recognize as self-structures the MHC determinants of the high responder

haplotype is consistent with the concept that the products of MHC-linked Ir genes are MHC-encoded cell surface determinants, such as the Ia antigens, which are recognized by T-helper cells as self structures.

Discussion

Self determinants are those MHC determinants which individual T cells recognize to respond to conventional (non-MHC) antigens, and are not necessarily the MHC determinants which the T cells themselves express. This study has further analyzed the role of the differentiation environment in dictating the MHC determinants competent T cells recognize as self structures. The experiments presented in this report have demonstrated that: (a) T-helper cells from fully allogeneic radiation bone marrow chimeras are functionally competent and recognize as self structures the allogeneic MHC determinants expressed by the chimeric host, but not the syngeneic MHC determinants expressed by the T cells themselves; (b) the recognition of allogeneic host MHC determinants as self structures results from the specific function of a radiation-resistant host element, and is not simply a consequence of the tolerization of T cell precursors to allogeneic host MHC determinants; and (c) the function of T-helper cells in responses regulated by MHC-linked Ir genes parallels their ability to recognize as self structures the MHC determinants of the high responder haplotype. Thus, the results of this study support the concept that the MHC determinants which T cells specifically recognize as self structures are those MHC determinants expressed on a radiation-resistant host element encountered by T cell precursors at a critical point in their differentiation (7).

Because these experiments demonstrate the existence of competent T cell function in $A \rightarrow B$ fully allogeneic chimeras, they directly conflict with the reported failure to generate virus-specific cytotoxic T lymphocytes (CTL) in fully allogeneic chimeras (7, 8, 12). The failure to detect competent virus-specific CTL in fully allogeneic chimeras therefore either reflects differences in the maturation requirements of T-helper cells versus CTL or results from specific features of the viral CTL assay itself. Because CTL also differentiate into functional competence in a fully allogeneic environment (A. Kruisbeek, R. J. Hodes, and A. Singer, manuscript in preparation), the possibility must be considered that the failure to generate virus-specific CTL from the spleens of fully allogeneic chimeras simply resulted from the failure to trigger the CTL precursors that were present, even though the lymphocytes from $A \rightarrow B$ chimeras were acutely transferred into irradiated and virally infected $(A \times B)F_1$ recipients to provide accessory cells expressing host type MHC determinants. Such a maneuver had been successful in activating the strain B-restricted CTL present in the spleens of $A \rightarrow A \times B$ semiallogeneic chimeras (7, 8). However, unlike spleen cells from $A \rightarrow A \times B$ semi-allogeneic chimeras which are tolerant to parental as well as F_1 MHC determinants, spleen cells from $A \rightarrow B$ fully allogeneic chimeras might be expected to be alloreactive against the unique hybrid determinants expressed on $(A \times B)F_1$ cells (22), because $A \rightarrow B$ chimeric lymphocytes have never been exposed or tolerized to such hybrid F_1 determinants. Indeed, as assayed in mixed lymphocyte reactions, spleen cells from $A \rightarrow B$ fully allogeneic chimeras are alloreactive against the hybrid MHC determinants expressed on $(A \times B)F_1$ cells (A. Singer, K. Hathcock, and R. J. Hodes, unpublished results). It is therefore possible that the alloreaction of $A \rightarrow B$ chimeric spleen cells against the hybrid determinants expressed in the irradiated F_1 short-term

host interfered with the ability of the F_1 presenting cells to activate the competent virus-specific CTL present in the allogeneic chimera spleen cell population. Thus, the failure to detect competent T cell function in fully allogeneic chimeras is probably a result of characteristics of the assays used to assess functional competence and not of the absence of competent T cells. Indeed, in addition to the present study, competent and host-restricted T cell function in fully allogeneic chimeras has also been reported in other functional systems as well (23, 24).

The restricted self recognition of host MHC determinants by T cells from fully allogeneic $A \rightarrow B$ chimeras resulted in the ability of strain A cells to recognize allogeneic B MHC determinants, but the failure to recognize syngeneic A MHC determinants. In contrast, the restricted self recognition of host MHC determinants by T cells from $A + B \rightarrow A$ double donor chimeras resulted in the ability of strain A T cells to recognize syngeneic A MHC determinants but the failure to recognize allogeneic B determinants. Thus, experiments with double donor chimeras demonstrated that tolerization of T cell precursors to allogeneic MHC determinants was not sufficient for those determinants to be recognized as self structures and suggested that the selection of MHC determinants to be recognized as self structures is a function of a specific radiation resistant host element, such as the thymus. These results are compatible with some acute depletion studies (25, 26) but conflict with others in which strain A lymphocyte populations that had been depleted *in vitro* of anti-B alloreactivity still contained T cells that could recognize allogeneic B MHC determinants as self structures (14–17). The difference in results between the double donor chimera experiments and those latter acute-depletion experiments may reflect real differences in the self recognition repertoires of strain A T cells that matured in an A thymus whose precursors were tolerized to allogeneic B MHC determinants either before or concomitant with the generation of their self recognition repertoire compared with strain A T cells that had also matured in an A thymus but had not been tolerized to allogeneic B MHC determinants until after their self recognition repertoire had been established. One way of understanding why the self recognition repertoires in these two cases would be different is to hypothesize that the MHC-specific self recognition repertoire is highly degenerate such that the same receptors which specifically recognize A MHC determinants as self structures can also cross-reactively recognize other MHC determinants as self structures as well. In other words, the self recognition repertoire can be conceived as consisting of two distinct receptor specificities: (a) a direct specificity for the self recognition of thymic expressed MHC determinants, and (b) cross-reactive specificities that fortuitously recognize third-party MHC determinants. However, tolerization of precursor cells to the MHC determinants present in their differentiation environment results not only in the deletion of clones alloreactive to those MHC determinants but also in the deletion of clones cross-reactive to those MHC determinants. Because precursor cells are tolerized to both donor and host MHC determinants, the cross-reactive repertoire is limited to the cross-reactive recognition of only third-party MHC determinants and not those of either the donor or host. Consequently mature T cells populations express a self recognition repertoire specific for the recognition of thymic MHC determinants and cross-reactive for the self-recognition of third-party, i.e., neither donor nor host, MHC determinants. From this perspective, the difference in results between chimera experiments and acute-depletion experiments results from the fact that chimera experiments

focus on the direct specificity of the self recognition repertoire, whereas acute depletion experiments focus on the cross-reactivity of that same repertoire.

The T cell self recognition repertoire has so far been considered in terms of its recognition of whole MHC haplotypes. However, in terms of Ir gene regulation the T cell self recognition repertoire might be better analyzed in terms of its recognition of individual MHC specificities that are encoded within a single MHC haplotype. Indeed, it can be hypothesized that all conventional antigens are presented by accessory cells and recognized by T cells in association with individual MHC specificities, though not necessarily those defined serologically (27, 28). In other words, conventional antigens such as KLH might be presented by strain A accessory cells in association with a variety of individual A specificities (e.g., a_1 , a_2 , a_4) so that the only T cells that respond to KLH presented by strain A accessory cells are those with receptors capable of recognizing both KLH and the individual MHC specificities a_1 , a_2 , or a_4 . However, because the self recognition repertoire is degenerate, it is possible that T cells that recognize a particular specificity (e.g., a_1) as a self recognition structure might incidentally alloreact against another MHC specificity (e.g., a_4) that is also encoded within the A haplotype. The tolerization of precursor clones to a_4 then coincidentally results in the deletion of clones specific for the self recognition of a_1 . As a result, there will be a mini-hole in the self recognition repertoire specific for the self recognition of a_1 expressed by all T cell populations whose precursors were tolerized to A MHC determinants. Because all precursor clones that utilized a_1 as a self recognition specificity were deleted regardless of their antigen specificity, all T cell populations tolerant to A will be unable to recognize and respond to any conventional antigen presented in association with a_1 . However, if an antigen such as (T,G)-A-L is presented by strain A accessory cells in association with a single self specificity and that self specificity happens to be a_1 , all the T cell populations that are tolerant to A will be unresponsive to strain A accessory cells presenting (T,G)-A-L. Even though these T cell populations would be unresponsive to any antigen presented in association with a_1 , the nonresponsiveness of these T cells to strain A accessory cells appears to be specific for (T,G)-A-L because: (a) multi-determinant antigens such as KLH are presented by strain A accessory cells in association with many specificities encoded within the A haplotype in addition to a_1 , and (b) other simple antigens that might also be presented by strain A accessory cells in association with only a single MHC specificity might be presented in association with a self-specificity other than a_1 . Thus, even though Ir gene regulation appears to be exquisitely antigen specific, it can result from antigen-nonspecific deletions in the T cell self recognition repertoire. This antigen-nonspecific clonal deletion model for Ir gene regulation is consistent with the dominant inheritance pattern of Ir gene-regulated responsiveness in (nonresponder \times responder) F_1 individuals because the tolerization of (A \times B) F_1 precursors to both parental MHC haplotypes (i.e., tolerization to specificities a_1 - a_n and b_1 - b_n) would coincidentally result in the deletion of precursors specific for the self recognition of low responder specificity a_1 but not the deletion of precursors specific for the self recognition of the high responder specificity (e.g., b_1) which is utilized by high responder parent B accessory cells for the presentation of (T,G)-A-L. This antigen-nonspecific clonal deletion model is similar to another model which also proposed that Ir gene-regulated nonresponsiveness occurs as a consequence of tolerization to the low responder haplotype (29), but significantly extends this other model by

proposing a mechanism by which antigen-nonspecific clonal deletions can result in apparent antigen specificity.

In summary, the experiments presented in this report demonstrate that the MHC determinants which T cells specifically recognize as self determinants are those expressed on a radiation-resistant host element. It is suggested that the self recognition repertoire is degenerate in that T cells can cross-reactively recognize MHC determinants that were not expressed in their differentiation environment, but that such cross-reactive self recognition does not include MHC determinants to which the precursor T cells were tolerized. Furthermore, it is suggested that antigen-specific Ir gene regulation of immune responses may derive from the existence of antigen-nonspecific mini-holes in the self recognition repertoire.

Summary

The specificity of the self-recognition repertoire in fully allogeneic ($A \rightarrow B$), semiallogeneic ($A \rightarrow A \times B$ and $A \times B \rightarrow A$), and double donor ($A + B \rightarrow A$) radiation bone marrow chimeras was assessed by the ability of their spleen cells to generate in vitro primary plaque-forming cell (PFC) responses to trinitrophenyl-keyhole limpet hemocyanin. In contrast to spleen cells from semiallogeneic and double donor chimeras, intact spleen cells from fully allogeneic $B10 \rightarrow B10.A$ and $B10.A \rightarrow B10$ chimeras were not capable of generating responses to trinitrophenyl (TNP)-keyhole limpet hemocyanin. However, cultures containing a mixture of both $B10 \rightarrow B10.A$ and $B10.A \rightarrow B10$ spleen cells did respond, demonstrating that all the cell populations required for the in vitro generation of T-dependent PFC responses were able to differentiate into functional competence in a fully allogeneic major histocompatibility complex (MHC) environment. The self recognition repertoire of T-helper cells from fully allogeneic $A \rightarrow B$ chimeras was determined to be specific for the recognition of host, not donor, MHC determinants in that they were able to collaborate with cells expressing only host MHC determinants but not with cells expressing only donor MHC determinants, even though the functional lymphocytes in these chimeras were shown to be of donor origin. Experiments utilizing double donor $A + B \rightarrow A$ chimeras further demonstrated that the ability of chimeric T cells to recognize allogeneic MHC determinants as self structures was a function of a radiation-resistant host element and not simply a consequence of the tolerization of T cell precursors to allogeneic MHC determinants, because strain A lymphocytes isolated from $A + B \rightarrow A$ chimeras were tolerant to both A and B MHC determinants but were restricted to the self recognition of syngeneic host type A MHC determinants.

Finally, the Ir gene phenotype expressed by $B10 \rightarrow B10.A$ and $B10.A \rightarrow B10$ chimeric lymphocytes was determined by their ability to function in the Ir gene controlled response to TNP-poly-L-(Tyr,Glu)-poly-D,L-Ala-poly-L-Lys [(T,G)-A-L]. The ability of lymphocytes to function in TNP-(T,G)-A-L responses was not determined by their genotype but rather paralleled the specificity of their self recognition repertoire for high responder ($H-2^b$) determinants. The possible degeneracy of the MHC-specific self recognition repertoire is discussed, and a model is proposed for Ir gene regulation in which expression of Ir gene function by lymphocytes is an antigen-nonspecific consequence of the specificity and cross-reactivity of their self recognition repertoire.

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