MAJOR HISTOCOMPATIBILITY COMPLEX-RESTRICTED SELF-RECOGNITION IN RESPONSES TO TRINITROPHENYL-FICOLL

Adaptive Differentiation and Self-Recognition by B Cells

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Most T lymphocytes can only recognize nominal antigens in the context of appropriate major histocompatibility complex (MHC)[1]-encoded determinants expressed on the surface of antigen-presenting cells (1). Such recognition of self-MHC determinants is a feature generally considered to be unique to T cells. Indeed, a great deal of evidence (2–4) has accumulated in support of the concept that, as they develop, T cells undergo a process termed "adaptive differentiation," such that the MHC polymorphisms that mature T cells can recognize for responses to nominal antigens are a function of the MHC determinants the T cells had encountered in their differentiation environment. Largely because the immunoglobulin expressed on the surface of B cells is capable of binding free antigen directly, it has been thought that self-recognition and adaptive differentiation are not features expressed by B lymphocytes. Contrary to this presumption, Katz and colleagues (5) reported experiments with B cells from radiation bone marrow chimeras that suggested that the collaborative phenotype expressed by these B cells was to some extent influenced by the environment in which the B cells had differentiated. Based on these experiments, Katz proposed that the concept of adaptive differentiation pertained not only to T cells but to B cells as well. Although the experimental results upon which the concept of B cell adaptive differentiation was based have been disputed (6), it was important to consider the possibility that activation of B cells, like activation of T cells, might involve recognition of self-MHC determinants.

However, the examination of self-recognition by B cells is prone to several complications. First, B cells, like T cells, are not triggered by nominal antigen alone, but require activation signals provided by other cells. Indeed, for responses to most antigens, the activation of B cells requires the elicitation of T cell help that itself is genetically restricted (7). Second, unlike T cells, which are predominantly activated by accessory cells, B cells can be activated by a variety of different mechanisms and a variety of different cellular activating factors, some of which might overcome requirements for self-MHC recognition by B cells even if such requirements otherwise existed. Finally, complicating matters still further, there exist in normal adult mice at

[1] *Abbreviations used in this paper:* C, complement; KLH, keyhole limpet hemocyanin; LPS, lipopolysaccharide; MHC, major histocompatibility complex; MLR, mixed lymphocyte reaction; PFC, plaque-forming cells; RAMB, rabbit anti-mouse brain serum; SAC, spleen adherent cells; sIg, surface immunoglobulin; TNP, trinitrophenyl; TNP-Ficoll, trinitrophenyl conjugate of Ficoll; TNP-KLH, trinitrophenyl conjugate of keyhole limpet hemocyanin; TNP-LPS, trinitrophenyl conjugate of lipopolysaccharide.

least two distinct B cell subpopulations (i.e. Lyb-5^+ and Lyb-5^-), which are quite distinct in their requirements for activation (8, 9).

To avoid as many of these potential complications as possible, the experiments presented in this report have focused on responses stimulated by "high" concentrations of trinitrophenyl (TNP)-Ficoll. Such responses have been shown to be essentially independent of a requirement for T cells (10, 11), require Ia^+ accessory cells (11–13), and are exclusively mediated by one B cell subpopulation, the Lyb-5^+ subset (8, 14). Consequently, responses stimulated by high concentrations of TNP-Ficoll offered a relatively simple and relatively well-defined system in which to specifically examine the existence of MHC restrictions between TNP-Ficoll-responsive B cells and accessory cells.

Materials and Methods

Animals. C57BL/10Sn (B10), B10.A, (B10 × B10.A)F_1, and B10.A(4R) adult male mice were obtained from The Jackson Laboratory, Bar Harbor, ME. B10 *nu/nu* mice were obtained from the National Institutes of Health Small Animal Section. B10.A(2R) mice were provided by Dr. David Sachs, NIH, and (B10.A × B10.A(2R))F_1 mice were provided by Dr. Gene Shearer, NIH, or were bred in our own animal colony.

Radiation Bone Marrow Chimeras. Radiation bone marrow chimeras are designated as bone marrow donor → irradiated recipient and were constructed as previously described (15). Briefly, recipient mice were lethally irradiated with 950 rad cesium and reconstituted 3–6 h later with 15×10^6 bone marrow cells that had been depleted of T cells by pretreatment with rabbit anti-mouse brain serum (RAMB) + complement (C). Radiation bone marrow chimeras were housed in a limited access facility in which the overall long-term survival of all chimera combinations was >60%. Chimeras were rested at least 2 mo before use. Spleen cells from individual chimeras were routinely typed by indirect immunofluorescence with strain-specific anti-H-2 reagents and were found to be virtually all (>95%) of donor origin. Chimeric spleen populations were also tested for proliferative responses in mixed lymphocyte reactions (MLR) and were found to be unresponsive to host- or donor-type-stimulating cells but were responsive to third-party allogeneic stimulating cells.

Antigens. Aminoethylcarboxymethyl$_{70}$-Ficoll (Ficoll) was obtained from Biosearch Laboratories, San Rafael, CA; keyhole limpet hemocyanin (KLH) (lot 530195) was obtained from Calbiochem-Behring Corp., American Hoechst Corp., San Diego, CA; and lipopolysaccharide (LPS) was the kind gift of Dr. John L. Ryan, Yale University, and was derived from the butanol extract of *Salmonella minnesota* R595 hexose-less mutant. Each antigen was conjugated with trinitrobenzene sulfonate, as previously described (11, 16), to obtain TNP-Ficoll, TNP-KLH, and TNP-LPS.

Cell Preparations

T CELLS. T cell enriched cell populations were obtained by passage of spleen cells over nylon fiber columns and collection of the nonadherent eluate.

B + ACCESSORY CELLS (T CELL-DEPLETED CELL POPULATIONS). Spleen cells were depleted of T cells by pretreatment with monoclonal anti-Thy-1.2 (the generous gift of Dr. Phil Lake, George Washington University, Washington, D. C.) + C. Where indicated, spleen cells were also treated with monoclonal anti-Lyt-1.2 (New England Nuclear, Boston, MA) + C.

ACCESSORY CELLS. Spleen adherent cells (SAC) were prepared as previously described (17). Briefly, spleen cells were allowed to adhere to glass for 2 h, harvested, depleted of T cells by treatment with RAMB + C, irradiated with 2,000 rad, and then pre-cultured overnight on a roller drum before addition to the antibody-forming cell cultures.

B CELLS (T CELL AND ACCESSORY CELL-DEPLETED CELL POPULATIONS). Spleen cells were first depleted of accessory cells by G-10 Sephadex passage (18) and then depleted of T cells as described above.

Culture Conditions for In Vitro Generation of Antibody-secreting Cells. 5×10^5 spleen cells or indicated numbers of spleen cell subpopulations were cultured in a volume of 200 μl containing

Eagles minimal essential medium supplemented with nutrients and 10% fetal calf serum for 4 d in a 5% CO_2-humidified air atmosphere as previously described (11, 16). Cells were cultured with 10–20 μg/ml final concentration of TNP-KLH, 2 μg/ml final concentration of TNP-LPS, or 10^{-2} μg/ml final concentration of TNP-Ficoll.

Assay for Plaque-forming Cells (PFC). Sheep erythrocytes were coupled with TNP by the method of Rittenberg and Pratt (19). Direct anti-TNP PFC were assayed by the slide modification of the Jerne hemolytic plaque technique (20). Unless otherwise indicated, each experimental point represents the geometric mean of three replicate cultures.

Mixed Lymphocyte Reactions (MLR). 4×10^5 responder spleen cells were cultured with 4×10^5 2,000 rad-irradiated stimulator spleen cells for 4 d. Cultures were pulsed with 1 μCi[^{3}H] thymidine 18 h before harvest. Each experimental point represents the geometric mean of three replicate cultures.

Anti-H-2K^k + C Treatment of Cells Before Culture. Monoclonal anti-H-2K^k reagent was a culture supernate of the hybridoma 11-4.1, described by Oi et al., (21) and obtained from the Cell Distribution Center of the Salk Institute (La Jolla, CA). 5×10^6 cells/ml were treated with a 1:4 dilution of the 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.

Anti-D^b + C Treatment of Cells After Culture. Anti-H-2^b reagent was a cytotoxic (B10.A × A/J)F_1 anti-B10 serum with an anti-D^b cytotoxic titer of 1:32 against B10.A(2R) spleen cells. After 4 d in culture and before assay for PFC, 10×10^6 cultured spleen cells were treated with a 1:15 dilution of this reagent in 2 ml 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. After this treatment, the surviving cells were assayed for their number of anti-TNP PFC.

Results

In Vitro Responses to "High" Concentrations of TNP-Ficoll Require Accessory Cells but Are Essentially Independent of T Cells. The in vitro generation of B cell responses to TNP-Ficoll has been shown to require accessory cells (10–12) that are Ia$^+$ (13), but it has been controversial whether these responses also require T cells (10–14, 22). To examine the cellular requirements for generating anti-TNP responses to "high" concentrations (10^{-2} μg/ml) of TNP-Ficoll, spleen cells from congenitally athymic nude mice that are deficient in T cells were further depleted of any residual T cells by treatment with anti-Thy-1.2 + C and anti-Lyt-1.2 + C and subsequently also depleted of accessory cells by G-10 Sephadex passage (Fig. 1). Such spleen cell populations did not respond to TNP-Ficoll; however, responses were generated upon the addition of SAC as accessory cells. It should be noted that the SAC populations were always depleted of T cells by RAMB + C, irradiated, and precultured overnight. The remaining viable cells in the SAC populations at the time of addition to the response cultures are

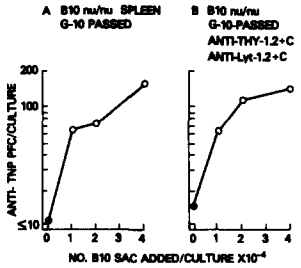

FIG. 1. Responses to high concentrations of TNP-Ficoll are T cell independent but accessory cell dependent. Graded numbers of B10 SAC were added to cultures of 5×10^5 B10 *nu/nu* spleen cells (A) that had been depleted of accessory cells, and (B) that had been depleted of both accessory cells and T cells, and stimulated with 10^{-2} μg/ml TNP-Ficoll. <10 PFC/culture were obtained in the absence of antigen.

markedly enriched in Ia^+ accessory cells but are devoid of T cells (23). Thus, these results suggest that the generation of in vitro PFC responses to "high" concentrations of TNP-Ficoll require responding B cells and adherent accessory cells but do not require T cells.

No Requirement Exists for MHC Homology between TNP-Ficoll-responsive B Cells and Accessory Cells. Because TNP-Ficoll responses did not require T cells, it was possible to examine the ability of TNP-Ficoll-responsive B cells to cooperate with MHC syngeneic and allogeneic accessory cells with only a minimal possibility of complicating T cell-mediated allogeneic effects. Unfractionated normal B10, B10.A, and B10 *nu/nu* spleen populations all responded to TNP-Ficoll (Fig. 2). In each case, purified B cells (spleen cells depleted of both T cells and accessory cells) did not respond (Fig. 2). However, responses were fully reconstituted by the addition of either syngeneic or allogeneic SAC. Although it is never possible to fully exclude the possibility that the activation of B cells by allogeneic SAC resulted from the existence of cryptic allogeneic effects, such a possibility seems highly unlikely, especially in the case of anti-Thy-1.2 + C-treated B10 *nu/nu* spleen cells (Fig. 2 B). Thus, these results suggest that no requirement exists for MHC homology between the B cells and accessory cells involved in the T-independent response to TNP-Ficoll.

Lymphocytes from Fully H-2 Allogeneic (A → B) Radiation Bone Marrow Chimeras Are MHC Restricted in Their Interaction with Accessory Cells for Responses to TNP-Ficoll. Although there was no requirement for MHC homology between the interacting B cells and accessory cells for responses to TNP-Ficoll, the possibility remained that activation of TNP-Ficoll-responsive B cells involved recognition of the MHC determinants expressed by the accessory cells. Indeed, it is quite clear from experiments using T_H cells from radiation bone marrow chimeras that MHC homology between interacting T_H cells and accessory cells is not required, even though activation of the T_H cells does require T_H cell recognition of the MHC determinants expressed by the accessory cells (15). For example, strain A T_H cells from fully H-2 allogeneic (A → B)

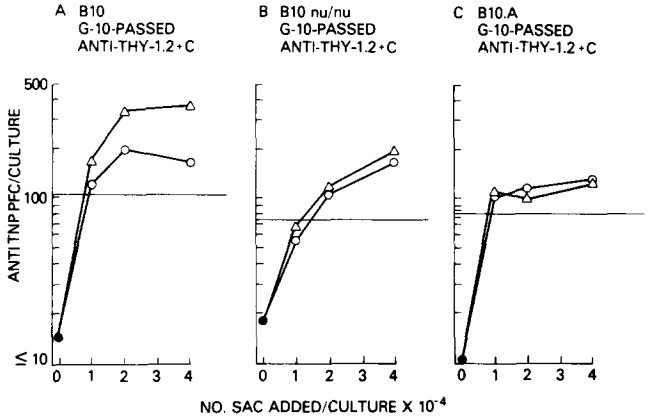

FIG. 2. Responses to TNP-Ficoll do not require MHC homology between B cells and accessory cells. Graded numbers of B10 or B10.A SAC were added to 5×10^5 spleen cells that had been depleted of both accessory cells and T cells from (A) B10, (B) B10 *nu/nu*, and (C) B10.A mice, and stimulated with 10^{-2} μg/ml TNP-Ficoll. The shaded line in each panel indicates the TNP-Ficoll responses of undepleted spleen cells. <10 PFC/culture were obtained in the absence of antigen. SAC: ○, B10; △, B10.A.

radiation bone marrow chimeras are unable to recognize and respond to nominal antigens presented by syngeneic accessory cells of donor type strain A but do recognize and respond to nominal antigens presented by allogeneic accessory cells of host type strain B (15). Consequently, to further explore the possibility that responsiveness to TNP-Ficoll might similarly require recognition of accessory cell-expressed MHC determinants, the ability of B cells from fully H-2 allogeneic radiation bone marrow chimeras to respond to TNP-Ficoll was examined.

First, the ability of unfractionated spleen cells from A → B and A → A × B chimeras to generate in vitro responses to TNP-Ficoll was determined. As can be seen in Table I, unfractionated spleen cells from fully allogeneic B10 → B10.A and B10.A → B10 chimeras failed to respond to TNP-Ficoll, even though spleen cells from B10 → (B10 × B10.A)F_1 and B10.A → (B10 × B10.A)F_1 chimeras did respond. The failure of spleen cells from fully allogeneic chimeras to respond to TNP-Ficoll was unlikely to reflect a failure of B cells to differentiate into functional competence in a fully allogeneic chimera because they did consistently respond to the TI-1 antigen TNP-LPS, one example of which is shown in Table I. Furthermore, the failure of spleen cells from fully allogeneic chimeras to respond to TNP-Ficoll was not due to the existence of either haplotype-specific or nonspecific suppressor cells because B10 → B10.A chimeric spleen cells did not suppress the ability of syngeneic B10 spleen cells to respond to TNP-Ficoll (Table I, experiment 2). To explore the possibility that the unresponsiveness to TNP-Ficoll of fully allogeneic chimeric spleen cells reflected the requirement for an MHC-restricted cell interaction, SAC of either the donor or host MHC haplotype were added to unfractionated spleen cells from B10 → B10.A and B10.A → B10 chimeras (Fig. 3). In the absence of added SAC, unfractionated spleen cells from neither chimera responded to TNP-Ficoll. The addition of SAC of donor type also had no effect. However, the addition of SAC of the host type fully restored

TABLE I

Spleen Cells from Fully Allogeneic Chimeras Do Not Respond to TNP-Ficoll

Experiment	Responding spleen cells‡	Anti-TNP PFC/culture*		
		No antigen	TNP-Ficoll§	TNP-LPS‖
1	B10.A → B10	0	4 (1.25)	190 (1.23)
	B10 → B10.A	0	4 (1.73)	ND¶
	B10.A → (B10 × B10.A)F_1	15 (1.37)	365 (1.07)	ND
	B10 → (B10 × B10.A)F_1	0	102 (1.17)	ND
	B10.A	0	248 (1.29)	199 (1.14)
	B10	7 (1.95)	293 (1.14)	135 (1.06)
2	B10	0	151 (1.05)	
	B10 → B10.A	0	1 (1.00)	
	B10 + B10 → B10.A	0	126 (1.37)	

* Geometric mean (SE) of triplicate cultures.

‡ 5×10^5 cells/culture. Mixtures of spleen cells contained equal numbers (2.5×10^5) of each cell population.

§ 10^{-2} μg/ml.

‖ 2 μg/ml.

¶ Not done.

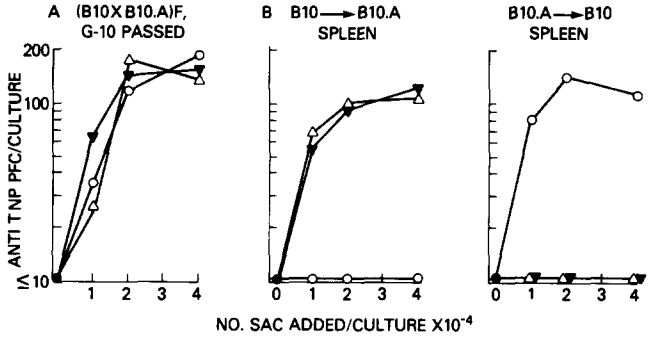

FIG. 3. Lymphocytes from fully allogeneic chimeras respond to TNP-Ficoll in the presence of accessory cells expressing the H-2K and/or I-A determinants of the chimeric host, but not of the chimeric donor. Graded numbers of B10, B10.A, or B10.A(4R) SAC were added to cultures of (A) (B10 × B10.A)F_1 spleen cells that had been depleted of accessory cells, (B) B10 → B10.A spleen cells, and (C) B10.A → B10 spleen cells, and stimulated with 10^{-2} μg/ml TNP-Ficoll. <10 PFC/culture were obtained in the absence of antigen. SAC: ○, B10; △, B10.A; ▼, B10.A(4R).

the ability of these allogeneic chimeric spleen cells to respond to TNP-Ficoll. Thus, B10 → B10.A spleen cells were able to respond to TNP-Ficoll in the presence of SAC from B10.A but not B10, whereas B10.A → B10 spleen cells were able to respond to TNP-Ficoll in the presence of SAC from B10 but not B10.A. Because the only variable in these cultures was the MHC haplotype of the added SAC, these results demonstrate that there exists an MHC-restricted lymphocyte-accessory cell interaction for the generation of responses to TNP-Ficoll.

To map the genes responsible for the MHC-restricted lymphocyte-accessory cell interaction, the ability of SAC from the H-2-recombinant strain B10.A(4R) to restore the TNP-Ficoll responsiveness of B10 → B10.A and B10.A → B10 chimeric spleen cells was also assessed. As can be seen in Fig. 3, SAC from B10.A(4R) behaved similarly to SAC from B10.A in that they restored the responsiveness of B10 → B10.A chimeric spleen cells but did not restore the responsiveness of B10.A → B10 chimeric spleen cells. These results demonstrate that the MHC-restricted cell interaction between lymphocytes and accessory cells for TNP-Ficoll responses is mediated by genes encoded in the left side (i.e., *K* and/or *I-A* region) of the *H-2* complex.

Although significant numbers of cells of host origin were not detected in these chimeras, it was formally possible that the responses generated by the chimeric spleen cells were mediated by residual cells of host origin. To rule out this possibility, spleen cells from B10 → B10.A chimeras were pretreated with monoclonal anti-K^k + rabbit C, a treatment that killed virtually all (>98%) B10.A spleen cells. Such treatment of the B10 → B10.A chimeric spleen cells did not diminish their ability to respond to TNP-Ficoll in the presence of host-type B10.A SAC and did not alter their unresponsiveness in the presence of B10 SAC (Fig. 4). The enhancement of responsiveness after treatment with anti-K^k + rabbit C that was observed in this experiment is a nonspecific effect consistently observed after pretreatment of spleen cells with rabbit C alone. Thus, the MHC-restricted cell interaction between chimeric lymphocytes and accessory cells for responses to TNP-Ficoll is mediated by cells of donor bone marrow origin and is induced by the host environment in which the lymphocytes had differentiated.

The MHC-restricted Interaction between Lymphocytes and Accessory Cells for the Generation of

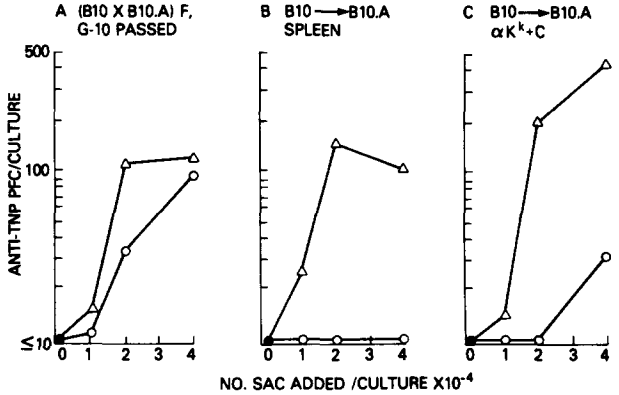

FIG. 4. TNP-Ficoll responsive lymphocytes from fully allogeneic chimeras are of donor bone marrow origin. Graded numbers of B10 or B10.A SAC were added to cultures of (A) (B10 × B10.A)F_1 spleen cells that had been depleted of accessory cells, (B) untreated B10 → B10.A spleen cells, and (C) anti-H-2K^k + C-treated B10 → B10.A spleen cells, and stimulated with 10^{-2} μg/ml TNP-Ficoll. <10 PFC/culture were obtained in the absence of antigen. SAC: ○, B10; △, B10.A.

TNP-Ficoll Responses Is Essentially Independent of T Cells. Although the interaction of chimeric lymphocytes with accessory cells for the generation of TNP-Ficoll responses was restricted by genes encoded in the left side of the *H-2* complex, the preceding experiments did not formally determine whether the MHC-restricted lymphocytes were T cells or B cells. Because responses to high concentrations of TNP-Ficoll can apparently be generated without T cells, it seemed likely that the observed genetic restrictions were also independent of T cells.

To examine this possibility, the ability of T-depleted chimeric spleen cells to interact with accessory cells was assessed. It can be seen in Fig. 5 that spleen cells from B10.A → B10 chimeras cooperated only with host type B10 SAC but not donor type B10.A SAC for responses to TNP-Ficoll as well as for responses to the T_H cell-dependent antigen TNP-KLH. After depletion of T cells by treatment with monoclonal anti-Thy-1.2 + C, the chimeric spleen cells failed to respond to TNP-KLH but continued to respond to TNP-Ficoll in the presence of B10 SAC. Thus, the genetically restricted interaction between chimeric lymphocytes and SAC for responses to TNP-Ficoll persisted even after the removal of most, if not all, T cells.

To determine whether the ability to observe genetic restriction between lymphocytes and accessory cells was unique to spleen cells from fully allogeneic chimeras, the ability of spleen cells from F_1 → parent chimeras to interact with SAC was next examined. Spleen cells from normal F_1 and F_1 → parent chimeras were first depleted of accessory cells by G-10 Sephadex passage and then depleted of T cells by sequential treatment with anti-Thy-1.2 + C and anti-Lyt-1.2 + C. It can be seen in Fig. 6 that without added SAC, none of the F_1 B cell populations responded significantly to TNP-Ficoll. Upon the addition of SAC, all three F_1 B cell populations responded. However, the response of (B10 × B10.A)F_1 → B10 B cells was much greater upon the addition of B10 SAC than upon the addition of B10.A SAC, whereas the response of (B10 × B10.A)F_1 → B10.A B cells was much greater upon the addition of B10.A SAC than upon the addition of B10 SAC (Fig. 6). Thus, these results demonstrate that the genetically restricted lymphocyte-accessory cell interactions that are involved in

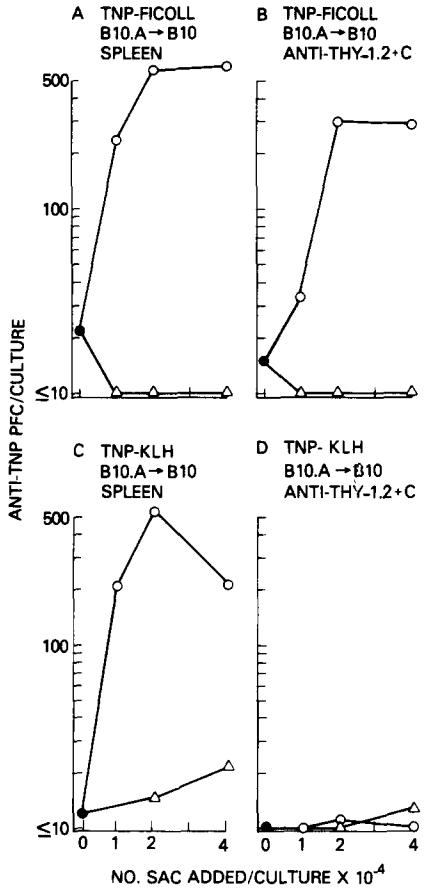

FIG. 5. Depletion of T cells from the spleens of fully allogeneic chimeras does not alter the presence of lymphocyte-accessory cell MHC restrictions for TNP-Ficoll. Graded numbers of B10 or B10.A SAC were added to cultures of (A, C) B10.A → B10 spleen cells and (B, D) T cell-depleted B10.A → B10 spleen cells, and stimulated with TNP-Ficoll (A, B) or TNP-KLH (C, D). <10 PFC/culture were obtained in the absence of antigen. SAC: ○, B10; △, B10.A.

responses to TNP-Ficoll can be observed for lymphocytes from F_1 → parent as well as from A → B chimeras. Furthermore, these results demonstrate that the MHC-restricted interaction between chimeric lymphocytes and accessory cells can be observed even when the chimeric lymphocyte populations had been vigorously depleted of T cells.

The Addition of F_1 T Cells Does Not Overcome the Preference of Chimeric Lymphocytes for Accessory Cells Expressing Host-Type MHC Determinants. Although they highly suggest that TNP-Ficoll responsive chimeric B cells are MHC restricted in their interaction with accessory cells, the preceding experiments have not excluded the possibility that responses to high concentrations of TNP-Ficoll require a small number of T cells that are present in *nu/nu* mice, that are resistant to anti-T cell treatments, and that are MHC restricted in their interactions with accessory cells. To examine this possibility, T cell addback experiments were performed. If the failure of B10.A → B10 chimeric spleen cells to respond to TNP-Ficoll in the presence of B10.A SAC was only due to

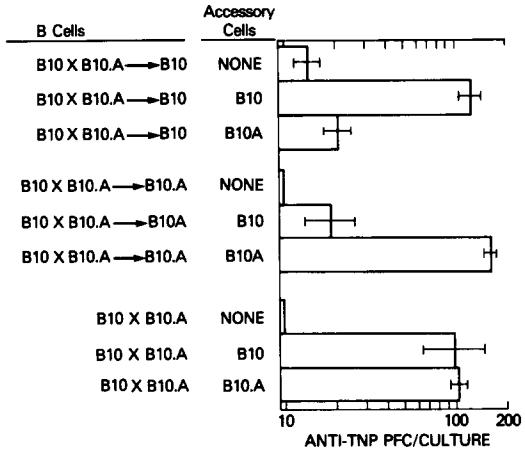

FIG. 6. TNP-Ficoll-responsive B cells from $F_1 \rightarrow$ parent chimeras are restricted in their interaction with accessory cells. 4×10^4 SAC from B10 or B10.A were added to the indicated spleen cell populations that had been G-10 passed and pretreated sequentially with anti-Thy-1.2 + C and anti-Lyt-1.2 + C, and stimulated with 10^{-2} μg/ml TNP-Ficoll. <10 PFC/culture were obtained in the absence of antigen.

the absence of T cells capable of recognizing H-2^a determinants expressed by accessory cells, then the addition of (B10 × B10.A)F_1 T cells that are capable of recognizing H-2^a SAC should fully restore the ability of B10.A → B10 chimeric spleen cells to respond to TNP-Ficoll in the presence of B10.A SAC. On the other hand, if lymphocytes other than T cells, e.g., B cells, from B10.A → B10 mice were unable to recognize H-2^a SAC, the addition of (B10 × B10.A)F_1 T cells would fail to activate B cells from B10.A → B10 chimeras for responses to TNP-Ficoll in the presence of B10.A SAC.

The mean of the results of six such experiments is shown in Fig. 7. As before, in the absence of any added T cells, T cell-depleted B10.A → B10 chimeric spleen cells failed to respond to TNP-Ficoll in the absence of added SAC or upon the addition of B10.A SAC, but did respond upon the addition of B10 SAC. The responses generated in the presence of B10 SAC were unaffected by the addition of F_1 T cells (Fig. 7). More importantly, the addition of relatively large numbers (12.5×10^4) of F_1 T cells had only a small effect on the inability of chimeric spleen cells to respond in the presence of added B10.A SAC. It should be noted that similar numbers of F_1 T cells were able to fully reconstitute the T-dependent responses of normal parental B + accessory cells to TNP-KLH (data not shown). The marginal responses observed with large numbers of F_1 T cells in the presence of donor type B10.A SAC were only inconsistently observed and are of unclear significance. In any event, it should be noted that the numbers of added T cells required to generate even small responses to TNP-Ficoll in the presence of B10.A SAC were far in excess of the numbers of T cells that could possibly have contaminated the chimeric B cell populations after T cell depletion maneuvers, so that the observed preference for B10 SAC expressed by B10.A → B10 T-depleted cell populations cannot be explained solely by the absence of nylon-nonadherent T cells capable of recognizing TNP-Ficoll presented by B10.A SAC. Rather, these results demonstrate that there exists an MHC-restricted cell interaction between a nylon-adherent cell, e.g., a B cell, and an accessory cell for

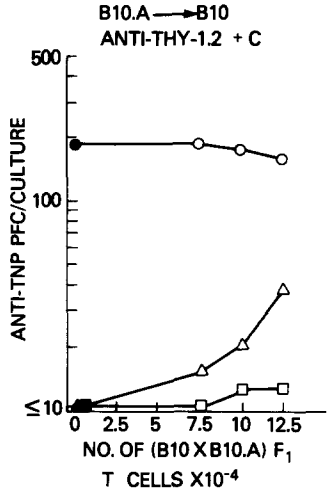

FIG. 7. F_1 T cells do not overcome the marked preference for host-type accessory cells of B + accessory cell populations from fully allogeneic chimeras. Mean of six consecutive experiments in which graded numbers of (B10 × B10.A)F_1 T cells were added to cultures which contained 4×10^5 T cell-depleted B10.A → B10 spleen cells and either no SAC or 4×10^4 SAC from B10 or B10.A, and stimulated with 10^{-2} μg/ml TNP-Ficoll. <10 PFC/culture were obtained in the absence of antigen. Added accessory cells: ○, B10; △, B10.A; □, none.

responses to TNP-Ficoll.

TNP-Ficoll Responsive B Cells from Fully Allogeneic Chimeras Fail to Be Activated in the Same Cultures That Activate "Syngeneic" B Cells from Normal Mice. Although the experiments presented above suggested that TNP-Ficoll-responsive chimeric B cells are genetically restricted in their interaction with accessory cells, the possibility remained that the chimeric restrictions were mediated by nylon-adherent T cells that would not have been present in the "unrestricted" nylon-nonadherent T cell populations that had been titrated into the cultures. Consequently, an experimental protocol was devised that would distinguish between the alternative possibilities that (*a*) the chimeric restrictions are entirely due to restricted self-recognition by chimeric T cells of accessory cell MHC determinants or that (*b*) TNP-Ficoll responsive chimeric B cells are themselves restricted in their recognition of accessory cell MHC determinants. These two alternative possibilities are schematically represented in Fig. 8 and predict two distinct experimental outcomes: (*a*) nonrecognition model: TNP-Ficoll-responsive B cells do not recognize accessory cell MHC determinants and do not undergo adaptive differentiation, so that strain A B cells from A → B chimeric mice are indistinguishable from strain A B cells from normal strain A mice. Thus, the chimeric restrictions observed for TNP-Ficoll responses are entirely due to genetic restrictions mediated by T cells. Consequently, because the normal strain A T cells would be activated in co-cultures containing unfractionated spleen cells from both strain A normal mice and A → B chimeric mice, stimulation of the co-cultures with TNP-Ficoll would result in the activation of both the normal and chimeric B cells. (*b*) active recognition model: TNP-Ficoll-responsive B cells do recognize accessory cell MHC determinants, and this recognition is restricted by the host environment in which the B cells had differentiated, so that strain A B cells from A → B chimeric mice are not identical to strain A B cells from normal mice in that A → B chimeric

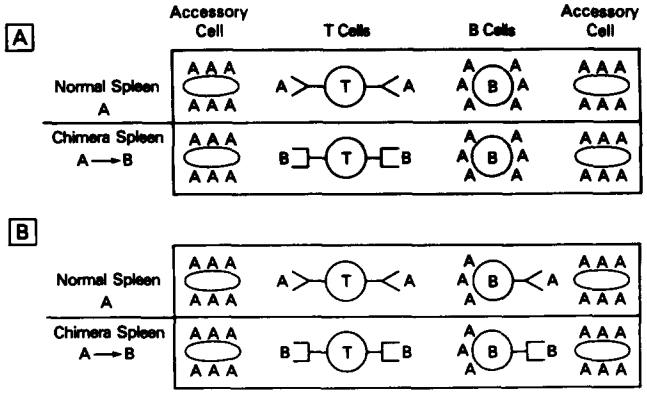

FIG. 8. Schematic representation of the cell populations present in co-cultures of spleen cells from strain A normal mice and A → B chimeric mice. (A) nonrecognition model: B cells do not recognize accessory cell MHC determinants so that strain A B cells from A → B chimeras are indistinguishable from B cells from normal strain A mice. (B) active recognition model: B cells do recognize accessory cell MHC determinants and adaptively differentiate in the chimeric host so that strain A B cells from A → B chimeras, in contrast to B cells from normal strain A mice, are unable to recognize strain A accessory cells.

B cells are unable to recognize the MHC determinants expressed by strain A accessory cells. Consequently, even though the normal strain A T cells would be activated in co-cultures containing unfractionated spleen cells from both strain A normal mice and A → B chimeric mice, stimulation of the co-cultures with TNP-Ficoll would only result in the activation of the normal B cells and not the chimeric B cells because these co-cultures are devoid of strain B accessory cells that are required to activate the A → B chimeric B cells.

To perform such a co-culture experiment, PFC that result from the activation of normal B cells must be distinguishable from PFC that result from the activation of chimeric B cells. Thus, (B10.A × B10.A(2R))F_1 mice were selected as the normal spleen cell source and B10.A → B10 chimeras were selected as the chimeric spleen cell source. (B10.A × B10.A(2R))F_1 spleen cells are identical to B10.A spleen cells, with the exception that they are heterozygous $D^{d/b}$, allowing them to be distinguished from B10.A by D^b-specific alloantisera. Because the MHC restrictions for TNP-Ficoll responses mapped to genes encoded within the *K* or *I-A* regions, heterozygosity in *H-2D* should not affect any of the relevant cell interactions. Furthermore, (B10.A × B10.A(2R))F_1 spleen cells should be genetically tolerant to $H\text{-}2^a$, and B10.A → B10 chimeric spleen cells should be tolerant to (B10.A × B10.A(2R))F_1 because the chimeric spleen cells are themselves $H\text{-}2^a$ and have matured in a B10 ($H\text{-}2^b$) host. That these two cells populations are in fact mutually tolerant is demonstrated in Table II.

Unfractionated spleen cells from (B10.A × B10.A(2R))F_1 and B10.A → B10 were consequently co-cultured together with TNP-Ficoll for 4 d. Before being assayed for numbers of anti-TNP PFC, the cultured cells were treated with C alone or anti-D^b + C to eliminate the PFC that were derived from normal (B10.A × B10.A(2R))F_1 B cells (Table III). As can be seen in Table III, treatment of normal B10.A PFC with anti-D^b + C had no effect (group A), whereas treatment of (B10.A × B10.A(2R))F_1 PFC with anti-D^b + C eliminated virtually all PFC (group B). Anti-D^b + C treatment

TABLE II

*Spleen Cells from B10.A → B10 and (B10.A × B10.A(2R))F1 Mice Are Mutually Tolerant**

Responder spleen cells	Stimulator spleen cells				
	B10.D2	B10	B10.A‡	B10.A → B10	(B10.A × B10.A(2R))F1§
			Δ *cpm* × 10^{-3}‖		
B10.A → B10	42.3	1.4	0.7	0	0.2
(B10.A × B10.A(2R)F1	38.4	23.4	0	0.1	0
B10.A → (B10 × B10.A)F1	16.4	1.1	0.1	ND¶	ND
B10	35.0	0	16.7	ND	ND
B10.A	30.6	17.5	0	ND	ND

* Mean of two experiments.

‡ H-2 haplotype (KABJECSD) is kkkkkddd.

§ H-2 haplotype (KABJECSD) is kkkkkdd$\frac{d}{b}$.

‖ Experimental cpm minus media cpm. Media cpm was <1,000 cpm in all groups.

¶ Not done.

TABLE III

TNP-Ficoll Responsive B Cells from B10.A → B10 Chimeric Spleens Are Not Triggered in Co-cultures with (B10.A × B10.A(2R))F1 Spleen Cells

Experiment	Group	Cultured spleen cells: Normal	Cultured spleen cells: Chimera	Antigen	Anti-TNP PFC*: C	Anti-TNP PFC*: Anti-D^b + C	Percent response remaining
1	A	B10.A‡	—	TNP-Ficoll	726	750	100
	B	(B10.A × B10.A(2R))F1‡	—	TNP-Ficoll	618	18	3
	C	(B10.A × B10.A(2R))F1	+ B10.A → B10	TNP-Ficoll	438	18	4
	D	(B10.A × B10.A(2R))F1	+ B10.A → B10	TNP-LPS	543	258	48
	E	(B10.A × B10.A(2R)F1	+ B10.A → (B10 × B10.A)F1	TNP-Ficoll	699	414	59
	F	1:1 Mix of PFC from groups A + B			702	351	50
2	A	B10.A	—	TNP-Ficoll	798	864	100
	B	(B10.A × B10.A(2R))F1	—	TNP-Ficoll	951	36	7
	C	(B10.A × B10.A(2R))F1	+ B10.A → B10	TNP-Ficoll	495	18	4
	D	(B10.A × B10.A(2R))F1	+ B10.A → B10	TNP-LPS	1,182	606	51
	E	1:1 Mix of PFC from groups A + B			837	333	40

* The indicated normal spleen cell populations or equal cell mixtures of normal and chimeric spleen cells were cultured for 4 d with the indicated antigen. At the end of this culture period, 10^7 cells of each group were treated with C alone or with anti-D^b + C and assayed for anti-TNP PFC.

‡ H-2 haplotype (KABJECSD): B10.A = kkkkkddd; (B10.A × B10.A(2R))F1 is kkkkkdd$\frac{d}{b}$

of a mixture of PFC derived by individually culturing B10.A and (B10.A × B10.A)2R))F1 resulted in the elimination of 50% of the PFC (Table III, experiment 1F, experiment 2 E), consistent with the treatment having only eliminated PFC derived from (B10.A × B10.A(2R))F1 B cells. Nevertheless, it was still possible that PFC derived from B10.A → B10 B cells when co-cultured with (B10.A × B10.A(2R))F1 spleen cells might passively acquire D^b alloantigen during the co-culture and be susceptible to lysis by anti-D^b + C treatment. To examine this possibility, B10.A → B10 chimeric spleen cells that are responsive to the TI-1 antigen TNP-LPS (Table I) were co-cultured with (B10.A × B10.A(2R))F1 spleen cells and stimulated with TNP-LPS. As can be seen in Table III (group D), anti-D^b + C treatment of these PFC only resulted in the elimination of ~50% of the response, consistent with the interpretation that even after co-culture, only PFC derived from (B10.A × B10.A(2R))F1 B cells

were sensitive to anti-D^b + C treatment. Thus, these control groups demonstrated that anti-D^b + C treatment of PFC would cytotoxically eliminate D^b bearing PFC derived from (B10.A × B10.A(2R))F_1 B cells but would not effect PFC derived from B10.A → B10 B cells.

The critical experimental group in Table III is group C, in which (B10.A × B10.A(2R))F_1 spleen cells were co-cultured with B10.A → B10 spleen cells and stimulated with TNP-Ficoll. It can be seen in Table III, group C that these co-cultures responded to TNP-Ficoll and that 96% of the PFC were sensitive to anti-D^b + C and, hence, were almost entirely derived from normal (B10.A × B10.A(2R))F_1 B cells. In other words, as predicted by the active recognition model, B10.A → B10 B cells were not triggered to respond to TNP-Ficoll, even though normal B cells in the same cultures were triggered to respond to TNP-Ficoll. It was not possible to add B10 SAC into the co-cultures to activate the B10.A → B10 chimeric B cells to respond to TNP-Ficoll because (B10.A × B10.A(2R))F_1 spleen cells are not tolerant to B10 SAC. However, these same B10.A → B10 B cells were also cultured individually and did respond to TNP-Ficoll upon the addition of B10 SAC (Fig. 3). To demonstrate that the failure of the B10.A → B10 chimeric B cells to respond in these co-cultures was specifically a consequence of their having differentiated in a fully allogeneic host environment, B10.A → (B10 × B10.A)F_1 chimeric spleen cells were also co-cultured with normal (B10.A × B10.A(2R))F_1 spleen cells and stimulated with TNP-Ficoll (Table III, experiment 1 E). B10.A → (B10 × B10.A)F_1 chimeric spleen cells are *H-2*^{a} but differ from B10.A → B10 chimeric spleen cells in the host environment in which they had differentiated. Because parent → F_1 chimeric spleen cells are able to respond to TNP-Ficoll without requiring the addition of any added accessory cells (Table I), it would be expected that parent → F_1 chimeric B cells would be activated in the co-cultures. Indeed, they were activated, as evidenced by the fact that 59% of the PFC were insensitive to anti-D^b + C treatment (Table III, experiment 1 E).

Thus, these co-culture experiments directly demonstrate that *H-2*^{a} B cells, which differentiated in different host environments, differ in their MHC requirements for activation in TNP-Ficoll responses. This conclusion is independent of any presumptions regarding the role of T cells in the generation of TNP-Ficoll responses because, if T cells are required, their function would have been provided by the (B10.A × B10.A(2R))F_1 T cells present in the co-cultures. Indeed, these results are incompatible with the nonrecognition model and are precisely those predicted by the active recognition model. Thus, it is concluded from these results that B cells recognize accessory cell MHC determinants for TNP-Ficoll responses and that their recognition of different MHC haplotypes is influenced by the host environment in which the B cells had differentiated.

MHC Restriction Paradox. The conclusion from the chimera experiments that TNP-Ficoll-responsive B cells recognize accessory cell MHC determinants appears to conflict with the observation that TNP-Ficoll-responsive B cells from normal mice are able to cooperate with both syngeneic and allogeneic accessory cells (Fig. 2). This apparent paradox is illustrated in Table IV, in which the ability of purified B cells from normal and fully allogeneic chimeric mice to cooperate with syngeneic and allogeneic SAC for responses to TNP-Ficoll was compared. In these experiments, the purified B cell populations were spleen cells that had been passed over G-10 Sephadex and treated with anti-Thy-1.2 + C. As can be seen in Table IV, B cells from normal

TABLE IV

MHC Restriction Paradox: Comparison of the Response Phenotype of B Cells from Normal Mice and Fully Allogeneic Chimeras for Responses to TNP-Ficoll

Experiment	Responding B cells‡	Accessory cells*			
		None	B10	B10.BR	B10.D2
		Anti-TNP PFC/culture§			
1	B10.BR	19 (1.05)	187 (1.11)	191 (1.05)	442 (1.08)
	B10.BR → B10	0	124 (1.32)	11 (1.56)	94 (1.20)
	B10	19 (1.56)	90 (1.35)	62 (1.22)	126 (1.16)
	B10 → B10.BR	0	3 (1.96)	101 (1.15)	106 (1.71)
		None	B10	B10.A	
2	B10.A	5 (1.48)	131 (1.19)	124 (1.09)	
	B10.A → B10	1	442 (1.08)	7 (1.84)	
	B10	0	101 (1.14)	79 (1.25)	
	B10 → B10.A	0	0	137 (1.31)	

* 4×10^4 SAC added per culture.

‡ G-10 passed and anti-Thy-1.2 + C-treated spleen cells.

§ Geometric mean (SE) of triplicate cultures. <10 PFC/culture were obtained in all groups in the absence of antigen.

mice cooperated with both syngeneic and allogeneic SAC, whereas genetically identical B cells from fully allogeneic chimeras cooperated with allogeneic host type and third-party SAC but failed to cooperate with syngeneic donor type SAC. Thus, the most apparent difference between B cells that had matured in a fully allogeneic chimeric environment and B cells that had differentiated in a normal syngeneic environment is not their ability to cooperate with allogeneic host type and third-party SAC but rather their failure to cooperate with accessory cells expressing syngeneic MHC determinants.

Discussion

The present study was undertaken to examine the possibility that MHC-restricted self-recognition might not be a feature unique to T cells but might also be expressed by B cells. To do so, we made use of the observation that TNP-Ficoll-responsive B cells appear to be activated by a direct interaction with TNP-Ficoll-presenting accessory cells (11, 14). Thus, the possibility existed that the putative interaction between TNP-Ficoll responsive (i.e., Lyb-5^+) B cells and accessory cells might be MHC restricted. Responses to "high" concentrations of TNP-Ficoll were specifically used in the present study because a substantial proportion of such responses appeared to be independent of T cells and only required an interaction between TNP-Ficoll-responsive B cells and accessory cells. The results of the present study demonstrate that the activation of TNP-Ficoll-responsive B cells does not require MHC homology between the interacting B cells and accessory cells but, nevertheless, does require B cell recognition of accessory cell MHC determinants. The present study also demonstrates that the MHC haplotype of the maturation environment in which the B cells had differentiated importantly influences the MHC haplotypes that they are subsequently able to recognize.

The existence of an MHC-restricted interaction between TNP-Ficoll-responsive B

cells and accessory cells has important implications for our understanding of how such B cells are activated. The results presented here suggest that presentation of TNP-Ficoll by accessory cells leads to the recognition of accessory cell Ia determinants by TNP-Ficoll-responsive B cells. Such recognition then results in the elaboration by accessory cells of activating signals that, together with TNP-Ficoll, trigger the TNP-Ficoll-responsive B cells to secrete immunoglobulin. The recognition of accessory cell MHC determinants by TNP-Ficoll-responsive B cells might occur via a cell surface receptor that is distinct from their surface immunoglobulin (sIg) or, alternatively, might occur because the sIg expressed by TNP-Ficoll-responsive B cells is able to recognize a complex of TNP plus accessory cell Ia. A precedent for the latter mechanism has recently been reported (24, 25) in that secreted immunoglobulin was shown to be able to recognize a complex of nominal antigen and MHC.

The mechanism by which the host environment influences the ability of B cells to recognize self-MHC determinants is not answered in this study and remains obscure, as it does for T cells. It is possible that Lyb-5^+ B cells are "educated" by a specialized host element in a manner analogous to which T cells apparently are educated in the thymus (4). Alternatively, it is possible that T cells interact with and expand those developing B cells that express anti-MHC specificities identical to the anti-MHC specificities expressed by the T cells themselves, resulting in the "tandem differentiation" of T cells and B cells in a manner analogous to that recently proposed for T cells involved in T suppressor circuits (26).

The present study illustrates an apparent paradox between the ability to activate TNP-Ficoll-responsive B cells from normal mice and the ability to activate TNP-Ficoll-responsive B cells from fully allogeneic chimeric mice. The results of experiments with B cells from both normal and chimeric mice were consistent in that, in both cases, there was no requirement for MHC homology between TNP-Ficoll-responsive B cells and accessory cells. However, B cells from normal mice, as opposed to B cells from chimeric mice, were not limited by the MHC haplotypes of the accessory cells with which they could interact. Thus, there appears to be a paradox in that no MHC barrier to cooperation exists between accessory cells and TNP-Ficoll-responsive B cells from normal mice, whereas such a barrier does exist for cooperation between accessory cells and TNP-Ficoll-responsive B cells from chimeric mice. However, there does exist preliminary evidence that the activation of TNP-Ficoll-responsive B cells from even normal mice may involve recognition of accessory cell MHC determinants in that the activation of TNP-Ficoll-responsive normal B cells is blocked by Ia-specific antibodies (A. Singer, K. S. Hathcock, and R. J. Hodes, unpublished results). Consequently, normal B cells as well as chimeric B cells probably recognize accessory cell MHC determinants for TNP-Ficoll responses. If this is indeed the case, the paradox can be reduced to the observation that B cells from normal mice can recognize accessory cells of virtually all MHC haplotypes, whereas B cells from either A $\rightarrow$ B or $F_1 \rightarrow$ parent chimeras can recognize accessory cells of virtually all MHC haplotypes with the exception of accessory cells expressing MHC determinants that are unique to the donor haplotype. It should be noted that precisely the same paradox probably also exists for T cells because, under some circumstances, T cells from normal strain A mice appear able to recognize nominal antigens presented by allogeneic strain B accessory cells even though T cells from B $\rightarrow$ A and A $\times$ B $\rightarrow$ A chimeras cannot (27). Thus, the apparent MHC restriction paradox can be resolved by postulating (*a*) that

the absence of a requirement for MHC homology does not necessarily imply the absence of a requirement for MHC recognition, so that TNP-Ficoll-responsive B cells from both normal and chimeric mice recognize accessory cell MHC determinants, (*b*) that virtually all lymphocytes from both normal and chimeric mice express receptors that are specific for the self-recognition of host-type MHC determinants but that can cross-reactively recognize third-party MHC determinants (also called "aberrant recognition" [27]), and (*c*) that, in chimeric mice, there is a paucity of lymphocytes specific for host determinants that cross-reactively recognize uniquely donor-type MHC determinants because most were eliminated as a consequence of being tolerant to the donor haplotype. Thus, there are net positive selective pressures for lymphocytes specific for host-type MHC determinants (e.g., "education"), no selective pressures for lymphocytes specific for third-party MHC determinants, and only negative selective pressures for lymphocytes specific for uniquely donor-type MHC determinants (e.g., tolerance). Consequently, there appears to be a "hole" in the self-recognition repertoires of lymphocytes from A $\rightarrow$ B and $F_1 \rightarrow$ parent chimeras, such that these chimeras possess lymphocytes that are able to recognize host-type and third-party MHC determinants but are specifically deficient in lymphocytes that are able to recognize MHC determinants that are unique to the chimeric donor haplotype. A similar analysis has been previously suggested for T cells (7, 15, 28).

It is important to emphasize that the present study has specifically examined the existence of MHC restrictions regulating the interaction between TNP-Ficoll-responsive (i.e., Lyb-5^+) B cells and accessory cells under conditions in which such an interaction occurs. Clearly, MHC restrictions regulating the interaction of Lyb-5^+ B cells with accessory cells would not be observed in responses in which such a B cell-accessory cell interaction did not occur. It is not yet clear whether such MHC restrictions would be observed in responses of Lyb-5^+ B cells to T cell-dependent antigens because, under some conditions, T_H cells are capable of secreting B cell-activating factors that can activate Lyb-5^+ B cells directly, without requiring a B cell-accessory cell interaction (29). It is also important to emphasize that the present experiments have not examined the possibility that Lyb-5^- B cells can also recognize the MHC determinants expressed by those cells with which Lyb-5^- B cells interact. Because the activation of Lyb-5^- B cells involves a direct interaction with T_H cells (9, 29) but apparently does not involve a direct interaction with accessory cells (14), it is conceivable that Lyb-5^- B cells do recognize T cell MHC determinants. Indeed, an MHC-restricted interaction between Lyb-5^- B cells and T_H cells may be precisely what was observed by Katz and colleagues (5).

The present results differ somewhat from those recently reported in which the existence of an MHC-restricted interaction between B cells and accessory cells was examined in T-dependent immune responses. Nisbet-Brown and colleagues (30) demonstrated in an adoptive transfer system that there appeared to be an MHC-restricted interaction between the B cells and accessory cells present in the short-term irradiated host. Surprisingly, these investigators found no influence of the host environment in which the B cells had differentiated. Katz and Benacerraf (31) and Gorczynski and colleagues (32) have reported for B cell responses to T-dependent antigens that the MHC environment in which the B cells had been originally primed significantly affected the MHC haplotype of the cells with which they could subsequently interact. The influence of the host environment in which the B cells had

differentiated was not examined. Despite these differences, the results of these previous studies are consistent with the concept that, under conditions in which a B cell-accessory cell interaction occurs, that interaction is MHC restricted.

In conclusion, the present study supports the concept that B cells, like T cells, can undergo "adaptive differentiation." However, it is expected that self-recognition by B cells would only be observed in those cell-cell interactions in which the responding B cells directly participate. In this regard, the present study used an experimental system in which T cells played no detectable role. The effect that T cells and their soluble products can have on the self-recognition requirements of TNP-Ficoll-responsive B cells will be addressed in a subsequent report.[2]

Summary

The present study has examined the possibility that TNP-Ficoll-responsive B cells recognize the MHC determinants expressed by the accessory cells with which they interact for the generation of T cell-independent responses to "high" concentrations (10^{-2} μg/ml) of TNP-Ficoll. In experiments with B cells from normal mice, it was found that MHC homology between the TNP-Ficoll-responsive B cells and accessory cells was not required. Nevertheless, TNP-Ficoll-responsive B cells from both fully allogeneic (A → B) and F_1 → parent radiation bone marrow chimeras were triggered by accessory cells expressing host-type, but not uniquely donor-type, MHC determinants. The MHC gene products responsible for this apparent B cell-accessory restriction were encoded in the left side, i.e., the *K* and/or *I-A* region, of *H-2*. Such genetic restrictions were shown not to be imposed by the residual T cells contaminating the chimeric B cell populations because T cell reconstitution experiments using "unrestricted" F_1 T cells from normal mice did not fully overcome the marked preference of the chimeric B cells for accessory cells expressing appropriate (host-type) MHC determinants.

To directly determine whether TNP-Ficoll-responsive B cells from fully allogeneic chimeras are unable to recognize and cooperate with syngeneic strain A accessory cells, unfractionated spleen cells from A → B chimeras were co-cultured with unfractionated spleen cells from essentially syngeneic normal strain A mice. In such co-cultures, all the accessory cells express strain A MHC determinants, and all T cell requirements would be fulfilled by the T cells present in the normal strain A spleen cell population. After stimulation of the co-cultures with TNP-Ficoll, it was found that virtually all the PFC that had been generated in the co-cultures were derived from the normal B cell population, and essentially none were derived from the chimeric A → B B cell population. The failure of the chimeric B cells to be activated in such co-cultures was specifically due to their maturation in a fully allogeneic host environment because TNP-Ficoll-responsive B cells from A → (A × B)F_1 chimeric mice were successfully triggered in co-cultures with normal spleen cells. These experiments demonstrated that the co-culture conditions did fulfill the MHC restriction requirements for activating TNP-Ficoll-responsive strain A B cells that had matured in a syngeneic or semi-syngeneic differentiation environment, but did not fulfill the MHC restriction requirements for activating TNP-Ficoll-responsive strain

[2] Hodes, R. J., K. S. Hathcock, and A. Singer. Major histocompatibility complex-restricted self-recognition in responses to TNP-Ficoll. A novel cell interaction pathway requiring self-recognition of accessory cell H-2 determinants by both T cells and B cells. Manuscript submitted for publication.

A B cells that had matured in a fully allogeneic differentiation environment.

Taken together, these results demonstrate that (*a*) TNP-Ficoll-responsive B cells recognize the MHC determinants expressed by accessory cells, and (*b*) their MHC specificity is influenced by the MHC haplotype of the host environment in which the B cells had differentiated.

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