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

A Novel Cell Interaction Pathway Requiring Self-Recognition of Accessory Cell H-2 Determinants by Both T Cells and B Cells

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Most thymus-dependent (T) lymphocyte populations are restricted in their function by a requirement for recognition of "self" major histocompatibility complex (MHC)[1] gene products. Thus, T cells that mediate help (T_H) (1–5), antigen-specific proliferation (6, 7), cell-mediated lympholysis (8, 9), delayed-type hypersensitivity (10), and immune suppression (11) require recognition of MHC products expressed by the cells with which they interact. In addition to MHC restriction of T cell recognition, the possibility that B cells as well as T cells may be capable of MHC-restricted self-recognition has recently been evaluated (12–15). In a recent report (15), the possibility that trinitrophenyl (TNP)-Ficoll-responsive B cells are MHC restricted in their interaction with accessory cells was assessed under T-independent (TI) response conditions. In the absence of any detectable requirement for T cells, it was demonstrated that TNP-Ficoll-responsive B cells, as a consequence of their development in a fully MHC-allogeneic chimeric host environment, were unable to cooperate with accessory cells expressing syngeneic donor-type MHC determinants but could cooperate with accessory cells expressing allogeneic host-type MHC products. These findings raised a number of other questions. Although they demonstrated that B cells could express MHC-restricted self-recognition in the absence of T cells, these studies did not determine the effects that T cells or their soluble products could exert on this B cell function. Specifically, it remained undetermined whether restricted recognition by B lymphocytes of accessory cell MHC determinants could be expressed in a response that concomitantly required the function of T_H cells. Moreover, since it has previously been suggested that B cells can be directly activated by antigen in the presence of T cell-derived factors (16), there was the possibility that such T cell products might be capable of bypassing or overcoming any MHC recognition requirement for the activation of B cells. In the present report, conditions were first established in which primary antibody responses to limiting concentrations of TNP-

[1] *Abbreviations used in this paper:* ATS, anti-thymocyte serum; C, complement; Con A, concanavalin A; IL-2, interleukin 2; KLH, keyhole limpet hemocyanin; MHC, major histocompatibility complex; MLR, mixed lymphocyte response; PFC, plaque-forming cell; RAMB, rabbit anti-mouse brain serum; SAC, spleen adherent cells; SRBC, sheep erythrocytes; TD, T dependent; (T,G)-A--L, poly-L-(Tyr,Glu)-poly-D,L-Ala--poly-L-Lys; T_H, T helper; TI, T independent.

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re shown to be highly T cell dependent (TD). Under these conditions, it was demonstrated that T_H cell function did require T_H cell recognition of accessory cell MHC determinants but did not require T_H cell recognition of B cell MHC determinants. However, T_H cell activation of TNP-Ficoll-responsive chimeric B lymphocytes still required B cell recognition of self-MHC determinants expressed by accessory cells. Thus, these findings demonstrate the existence of a novel cell interaction pathway in which both TNP-Ficoll-reactive B cells and T_H cells express requirements for MHC-restricted self-recognition of accessory cells.

Materials and Methods

Animals. C57BL/10 (B10), B10.A, B10.BR, (B10 × B10.A)F_1, (B10 × B10.BR)F_1, and (C57BL/6 × A/J)F_1 (B6AF_1) mice were obtained from The Jackson Laboratory, Bar Harbor, ME. B10.A(2R) mice were provided by Dr. David Sachs, National Institutes of Health (NIH), and [B10.A × B10.A(2R)]F_1 mice were provided by Dr. Gene Shearer, NIH.

Radiation Bone Marrow Chimeras. Chimeras were prepared as previously described (5). 12–15 × 10^6 T cell-depleted donor bone marrow cells were injected intravenously into 950-rad-irradiated recipients and rested for 8–24 wk before use. Chimeric spleen cell populations were routinely H-2 typed by indirect immunofluorescence and were uniformly of donor origin without detectable (<5%) host cells. These populations were also tested for proliferative responses in mixed lymphocyte response (MLR) and were found to be unresponsive to host- or donor-type stimulating cells but responsive to third-party allogeneic stimulators.

Antigens. TNP-Ficoll was prepared as previously described (17). Sheep erythrocytes (SRBC) used as antigen were obtained from a single sheep (1245) maintained at the NIH Large Animal Holding Facility, Poolesville, MD.

Antisera. Cytotoxic (B10.A × A/J) anti-B10 serum was generously provided by Dr. David Sachs and had cytotoxic titers of 1:128 against B10 spleen cells, 1:32 against B10.A(2R), and was without significant activity against B10.A target cells. Monoclonal 11-4.1 culture supernatant was used as a cytotoxic anti-K^k reagent with a cytotoxic titer of 1:64 against B10.BR spleen cells (18). For elimination of K^k-bearing cells, this reagent was used in excess at a concentration of 1:2.

Cell Preparations. Sterile spleen cell suspensions and fractionated subpopulations of T cells, B cells, and accessory cells were prepared as described previously (19).

T Cells. Nylon-nonadherent spleen cells were used as a source of enriched T cells (19).

B Plus Accessory Cells. T cell-depleted (B plus accessory) cells were routinely prepared by treatment of spleen cells with cytotoxic monoclonal anti-Thy-1.2 plus complement (C) (16). In selected experiments, more exhaustive depletion was carried out by in vivo treatment of mice with 0.5 ml of a 1:10 dilution of rabbit anti-mouse thymocyte serum (ATS) (M. A. Bioproducts, Walkersville, MD), followed by sequential in vitro treatment with monoclonal anti-Thy-1.2 plus C and anti-Lyt-1.2 (New England Nuclear, Boston, MA) plus C.

B Cells. B cell populations depleted of both T cells and accessory cells were prepared by first passing spleen cells over Sephadex G-10 columns (19) and then treating the resulting cells with anti-Thy-1.2 plus C.

Spleen Adherent Cells (SAC). SAC as a source of accessory cells (19) were prepared by glass adherence of spleen cells, followed by recovery of adherent cells, treatment with a T cell-specific rabbit anti-mouse brain serum (RAMB) (19) plus C, irradiation with 2,000 rad, and overnight culture on a roller drum at 37°C before use.

T Cell Supernatants

FS6-14.13. The T cell hybridoma FS6-14.13 (FS6) was generously provided by Dr. John Kappler, National Jewish Hospital, Denver, CO (20). To generate supernatant, hybridoma cells were cultured for 24 h with 2.5 μg/ml concanavalin A (Con A) (Miles Laboratories, Inc., Elkhardt, IN). At the end of this culture period, 0.2 M alpha-methyl-D-mannoside (Sigma Chemical Co., St. Louis, MO) was added to prevent the mitogenic effects of residual Con A, and culture supernatant was filtered through a 0.45-μm filter.

SPLEEN SUPERNATANT. Con A supernatant from mouse spleen cells was prepared by culturing BALB/c spleen cells with Con A for 18–20 h as previously described (16). Alpha-methyl-D-mannoside was added as for the preparation of FS6 supernatant.

CLONE 16 AND CLONE 1A36. Clone 16 is a keyhole limpet hemocyanin (KLH)-specific and $I\text{-}A^b$-restricted T cell line of (B6 × C3H)F_1 origin (21), and clone 1a36 is a poly-L-(Tyr,Glu)-poly-D,L-Ala--poly-L-Lys [(T,G)-A--L]-specific and $I\text{-}A^b$-restricted T cell line of B6 origin (22). Supernatants were generated by stimulation of each clone with irradiated syngeneic spleen cells and 50 μg/ml of the appropriate antigen for 4 d and were generously provided by Dr. Minoru Shigeta, Dr. Masao Kimoto, and Dr. C. Garrison Fathman, Stanford University.

In Vitro Response Cultures. Primary antibody response cultures were carried out under conditions previously described (19). The indicated cell populations, antigen, and soluble factors were cultured in 200-μl flat-bottomed microtiter wells for 4 d at 37°C in 5% CO_2-air atmosphere. All cultures were carried out in triplicate. Cells were harvested at the end of the culture period, washed, and assayed for plaque-forming cell (PFC) response.

PFC Response. Direct (IgM) anti-SRBC or anti-TNP PFC responses were assayed as previously described (19). When PFC were being analyzed for *H-2* type, cells were treated at 5 × 10^6/ml with a 1:15 dilution of anti-H-2^b reagent at 37°C for 30 min, washed, exposed to a 1:6 dilution of rabbit C (Pel-Freeze Biologicals, Rogers, AR) for 30 min at 37°C, washed twice, and assayed for residual cell number and PFC activity.

Results

Antibody Responses to Limiting Concentrations of TNP-Ficoll Are T Cell Dependent. Although the generation of antibody responses to TNP-Ficoll has been most extensively characterized as being independent of a requirement for T cells (15, 17), recent studies (23, 24) have suggested that under certain experimental conditions, the generation of responses to TNP-Ficoll may be substantially T dependent. To study T cell influences on the activation of TNP-Ficoll-responsive B cells, experiments were carried out to establish conditions in which the T dependence of TNP-Ficoll responses would be clearly apparent. As previously reported, primary in vitro antibody responses to 10^{-2} μg/ml TNP-Ficoll were relatively T independent, showing little or no effect of T cell depletion when responding B10 spleen cells were treated with anti-Thy-1.2 plus C (Fig. 1). In contrast, responses to 10^{-3} μg/ml TNP-Ficoll were completely abrogated

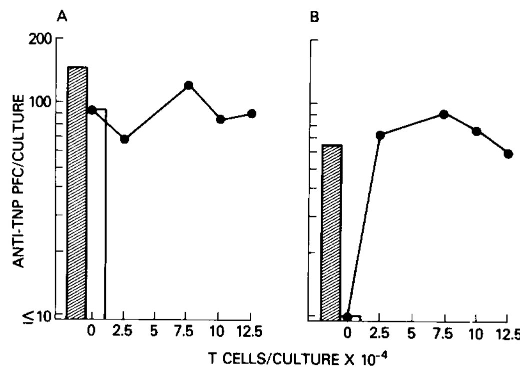

FIG. 1. The antibody response to limiting concentrations of TNP-Ficoll is T cell dependent. The responses of 5 × 10^5 untreated B10 spleen cells to either (A) 10^{-2} or (B) 10^{-3} μg/ml TNP-Ficoll are indicated by the hatched bars. Responses of 4 × 10^5 anti-Thy-1.2 plus C-treated B10 spleen cells to each dose of TNP-Ficoll are indicated by open bars. Titrated numbers of (B10 × B10.A)F_1 nylon-nonadherent spleen (T) cells were added to 4 × 10^5 anti-Thy-1.2 plus C-treated B10 spleen cells, and the responses to (A) 10^{-2} or (B) 10^{-3} μg/ml TNP-Ficoll were determined (●). Responses in the absence of antigen were <10 PFC/culture.

in the same T-depleted population and were fully reconstituted by the addition of small numbers of nylon-nonadherent B10 spleen (T) cells, thereby demonstrating a requirement for T cells in the generation of responses to "low" concentrations of TNP-Ficoll (Fig. 1). These latter conditions were therefore used to study the cell interaction requirements for T cell-dependent activation of TNP-Ficoll-responsive B cells.

T Cell-dependent Responses to TNP-Ficoll Require MHC-restricted Recognition of Accessory Cells by Chimeric Spleen Cells. It was previously demonstrated (15) that the generation of TI responses to higher concentrations of TNP-Ficoll required MHC-restricted recognition of accessory cells by responding spleen cells from fully allogeneic radiation bone marrow chimeras. To determine whether the TD responses to low concentrations of TNP-Ficoll were similarly restricted, the responses of fully MHC-allogeneic chimeric spleen cell populations were again studied. Untreated B10.A → B10 spleen cells were by themselves entirely unresponsive to 10^{-3} μg/ml TNP-Ficoll. However, the addition of host-type B10 SAC as a source of accessory cells permitted a vigorous response by these chimeric cells, whereas the addition of donor-type B10.A SAC was without effect (Fig. 2). The reciprocal B10 → B10.A chimeric spleen cells were similarly unresponsive and were competent to respond in the presence of host- (B10.A) but not donor-type (B10) SAC (data not shown). In each instance, the chimeric spleen cells were shown to be specifically unresponsive in MLR to both host- and donor-type stimulating cells, which indicates that the observed effects were not the result of allogeneic effects (data not shown). These findings demonstrated that one or more cell populations contained within chimeric spleens were, as a result of their chimeric maturation, restricted in their ability to cooperate with accessory cells expressing host- but not donor-type MHC determinants in TD responses to low concentrations of TNP-Ficoll.

T_H Cells for Responses to TNP-Ficoll Are MHC Restricted. The possibility was first evaluated that the T_H cells that are required for responses to low concentrations of

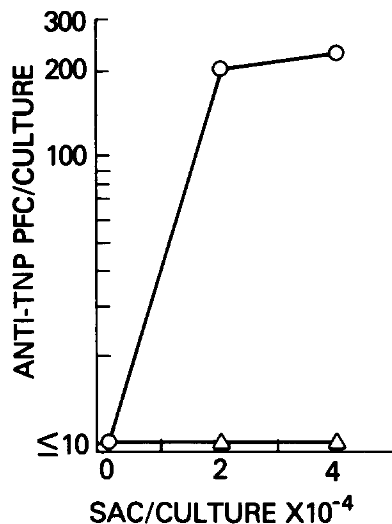

FIG. 2. T cell-dependent responses to TNP-Ficoll are MHC restricted for recognition of accessory cells. 5 × 10^5 B10.A → B10 spleen cells were cultured with the indicated numbers of B10 (○) or B10.A (△) SAC and assayed for responses to 10^{-3} μg/ml TNP-Ficoll.

TNP-Ficoll might be MHC restricted in their function. Nylon-nonadherent T cells from either F_1 → parent ($B6AF_1$ → B10 or $B6AF_1$ → B10.A) or allogeneic (B10.A → B10 or B10 → B10.A) chimeras were assessed for their ability to cooperate with T-depleted B10 or B10.A (B plus accessory) cells. Each chimeric T cell population cooperated efficiently only with B plus accessory cells of chimeric host origin, which demonstrated that the function of T_H cells in TNP-Ficoll responses was indeed MHC restricted (Fig. 3). Thus, these experiments demonstrate that the T_H cells that function in responses to the polysaccharide antigen TNP-Ficoll are similar to T_H cells that function in conventional TD responses to polypeptide antigens in terms of their requirement for MHC-restricted self-recognition.

T_H Cells for TNP-Ficoll Are Restricted in their Recognition of MHC Determinants Expressed by Accessory Cells but Not B Cells. To gain insight into the mechanisms by which T_H cells activate TNP-Ficoll-responsive B cells, it was next determined whether these T_H cells were required to recognize the MHC determinants expressed by accessory cells, B cells, or both. First, it was again demonstrated that chimeric B10.BR → B10 T cells cooperated with host (B10) but not donor (B10.BR) B plus accessory cells that had been rigorously depleted of T cells by a combination of in vivo pretreatment with ATS and in vitro treatment with anti-Thy-1.2 plus C and anti-Lyt-1.2 plus C (Fig. 4). However, when host-type B10 accessory cells (SAC) were added in culture, the B10.BR → B10 T cells were equally able to cooperate with B10 or B10.BR B plus accessory cells, which indicated that (*a*) the T_H cells were restricted in their recognition of MHC determinants expressed by accessory cells but not B cells, and (*b*) that for TD

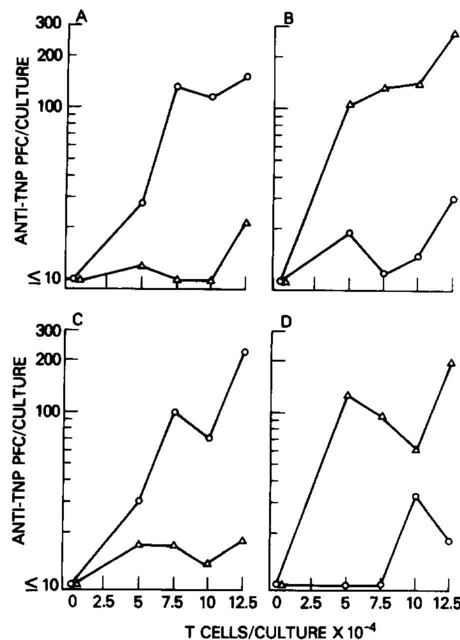

FIG. 3. T_H cell function is MHC restricted in T cell-dependent responses to TNP-Ficoll. Titrated numbers of (A) $B6AF_1$ → B10, (B) $B6AF_1$ → B10.A, (C) B10.A → B10, or (D) B10 → B10.A nylon-nonadherent spleen (T) cells were cultured with 4×10^5 anti-Thy-1.2 plus C-treated B10 (○) or B10.A (△) spleen B plus accessory cells and assayed for responses to 2×10^{-3} μg/ml TNP-Ficoll. Responses in the absence of antigen were <10 PFC/culture.

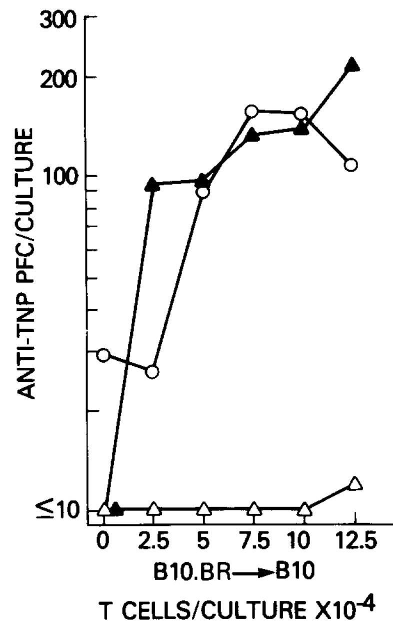

FIG. 4. Helper T cells are MHC restricted for recognition of accessory cells but not B cells for responses to TNP-Ficoll. Titrated numbers of nylon-nonadherent B10.BR → B10 spleen (T) cells were added to 4 × 10^6 anti-Thy-1.2 plus C- and anti-Lyt-1.2 plus C-treated spleen B plus accessory cells from in vivo anti-thymocyte pretreated B10 (○) or B10.BR (△) mice, or to a mixture of 4 × 10^5 B10.BR B plus accessory cells plus 4 × 10^4 B10 SAC (▲), and cultures were assayed for responses to 2 × 10^{-3} μg/ml TNP-Ficoll. Responses in the absence of antigen were <10 PFC/culture.

responses to TNP-Ficoll there was no requirement for MHC homology between B cells and accessory cells.

Soluble T Cell Products Are Capable of Overcoming the Requirements for MHC-restricted Cell Interactions in Responses to TNP-Ficoll. It has previously been shown (16, 21, 25, 26) for Lyb-5$^+$ B cell responses to polypeptide antigens that T_H cells are similarly MHC restricted in their recognition of MHC determinants expressed by accessory cells but not B cells. However, in such responses, T_H cell function can be replaced by antigen-nonspecific and MHC-unrestricted soluble products secreted by activated T cells (16, 26). To ascertain whether soluble T cell products could activate TNP-Ficoll-responsive B cells as well, the activities of two different T cell supernatant preparations were first assessed on B10 spleen cells that had been treated with anti-Thy-1.2 plus C. The T cell-depleted B10 spleen cells were unable to respond to 2 × 10^{-3} μg/ml TNP-Ficoll (Fig. 5). Titrated amounts were then added of supernatants from either the Con A-stimulated interleukin 2 (IL-2)-secreting hybridoma FS6 or from the antigen-stimulated KLH-specific T cell clone 16. These preparations did not themselves activate a PFC response in the absence of antigen, but each preparation fully reconstituted the responsiveness of T-depleted spleen cells to TNP-Ficoll (Fig. 5). The possibility that these factors were not in fact T cell replacing but rather acted upon residual T cells in the anti-Thy-1.2 plus C-treated populations was tested by examining the ability of these factors to also reconstitute responses to SRBC in vitro. Harwell et al. (20) have reported that in the absence of any residual T cells, hybridoma FS6 supernatant fails to reconstitute anti-SRBC responses even though heterogeneous supernatant from

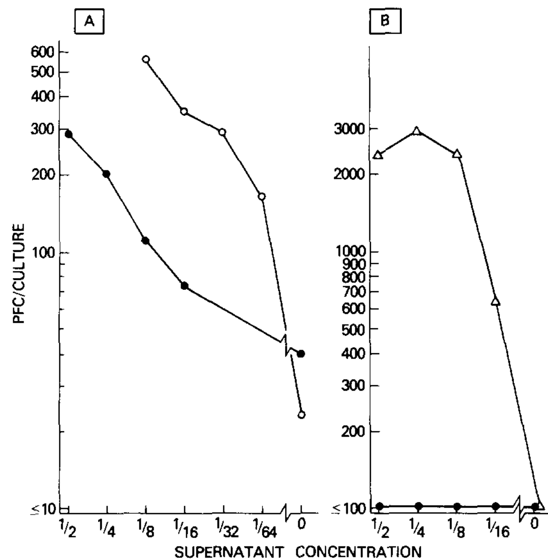

FIG. 5. Monoclonal T cell supernatants overcome T cell requirements for responses to TNP-Ficoll but not SRBC. 5×10^5 anti-Thy-1.2 plus C-treated B10 spleen cells were cultured for 4 d with (A) 2×10^{-3} μg/ml TNP-Ficoll or (B) 0.05% SRBC in the presence of titrated amounts of supernatant from clone 16 (○), hybridoma FS6 (●), or ConA-stimulated BALB/c spleen cells (△).

Con A-stimulated spleen cells does reconstitute such responses. In the present study, it was similarly found that even though hybridoma supernatant did reconstitute responses to TNP-Ficoll, it did not reconstitute responses to SRBC by the same T-depleted spleen cell population (Fig. 5). Thus, these results strongly suggest that the hybridoma or cloned T cell factors activate TNP-Ficoll-responsive B cells directly and not via effects on residual T cells.

One mechanism by which antigen and soluble T cell factors could activate TNP-Ficoll-responsive B cells directly is by circumventing any further requirement for either T cells or accessory cells. This possibility was tested by sequentially depleting B10 spleen cell populations of both accessory cells and T cells and then determining the ability of the purified B cell population to respond to 2×10^{-3} μg/ml TNP-Ficoll in the presence or absence of T cell supernatant factors. It was observed that such purified B cell populations were unresponsive to TNP-Ficoll alone but responded vigorously to TNP-Ficoll in the presence of soluble products of cloned T cells (Table I).

If such T cell factors did indeed directly activate the TNP-Ficoll-responsive B cells without any further requirement for cell interaction, it would be predicted that these factors would overcome any MHC restriction otherwise imposed upon the activation of TNP-Ficoll-responsive B cells. To evaluate this possibility, supernatants from either the hybridoma FS6 or the T cell clone 16 were added to cultures of chimeric B10 → B10.BR spleen cells and 2×10^{-3} μg/ml TNP-Ficoll. Chimeric spleen cells, which were otherwise responsive only in the presence of appropriate (*H-2^k*) accessory cells, were highly responsive to TNP-Ficoll in the presence of either of these T cell supernatants (Table II). These findings suggested either that these T cell factors were able to overcome the requirement for MHC-restricted cell interactions in these

TABLE I
Monoclonal T Cell Supernatants Overcome Both T Cell and Accessory Cell Requirements for TD Responses to TNP-Ficoll

FS6 hybridoma supernatant concentration^{-1}	TNP-specific PFC/culture*	
	B10 spleen G10 passed	B10 spleen G10 passed, anti-Thy-1.2 plus C
—	29 (1.80)	6 (1.49)
2	241 (1.13)	163 (1.15)
6	168 (1.11)	187 (1.21)
18	56 (1.47)	77 (1.10)
Clone 1a36 supernatant concentration^{-1}		
—	29 (1.80)	6 (1.49)
18	969 (1.11)	608 (1.05)

* 5 × 10^5 G10-passed or G10-passed and anti-Thy-1.2 plus C-treated B10 spleen cells were cultured for 4 d with 2 × 10^{-3} μg/ml TNP-Ficoll in the presence of the indicated T cell supernatant. Responses indicated represent the geometric mean (standard error) of triplicate cultures. Responses in the absence of antigen were <10 PFC/culture.

TABLE II
Monoclonal T Cell Supernatants Overcome Chimeric Restrictions for TD Responses to TNP-Ficoll

Supernatant concentration^{-1}	PFC/culture* B10 → B10.BR spleen	
	Hybridoma FS6	Clone 16
—	9 (1.78)	9 (1.78)
2	144 (1.15)	ND‡
4	192 (1.26)	ND
8	43 (1.15)	386 (1.15)
16	ND	226 (1.24)
32	ND	139 (1.11)
64	ND	46 (1.09)

* 5 × 10^5 B10 → B10.BR spleen cells were cultured for 4 d with 2 × 10^{-3} μg/ml TNP-Ficoll in the presence of the indicated T cell supernatant. Responses indicated represent the geometric mean (standard error) of triplicate cultures. Responses in the absence of antigen were <10 PFC/culture.
‡ Not done.

responses, or alternatively, that they were able to enhance the ability of chimeric B10 → B10.BR spleen cells to cooperate with small numbers of residual host type ($H\text{-}2^k$) accessory cells present in the chimeric population. To distinguish between these two possibilities, B10 → B10.BR spleen cells were first treated with a cytotoxic anti-K^k reagent plus C to eliminate any residual $H\text{-}2^k$ cells and then tested for their functional competence. These treated cells remained MHC restricted, cooperating with B10.BR but not B10 SAC for responses to TNP-Ficoll. Moreover, these restricted chimeric spleen cells were still activated by hybridoma FS6 supernatant for these responses (Table III). Thus, these results demonstrate that, in the presence of T cells factors,

TABLE III

Monoclonal T Cell Supernatants Activate TNP-Ficoll-responsive B Cells in the Absence of Host-Type Accessory Cells

FS6 supernatant concentration^{-1}	SAC	PFC/culture* B10 → B10.BR spleen	
		C	Anti-K^k plus C
—	—	9 (1.37)	18 (1.27)
—	B10	9 (1.10)	38 (1.04)
—	B10.BR	532 (1.04)	989 (1.18)
2	—	341 (1.14)	501 (1.13)
4	—	444 (1.11)	712 (1.16)

* 5 × 10^5 B10 → B10.BR spleen cells that had been pretreated with either C alone or with anti-K^k antibody plus C were cultured for 4 d with 2 × 10^{-3} μg/ml TNP-Ficoll in the presence of the indicated concentration of FS6 supernatant or in the presence of 4 × 10^4 B10 or B10.BR SAC as a source of added accessory cells. Responses indicated represent the geometric mean (standard error) of triplicate cultures. Responses in the absence of antigen were <10 PFC/culture.

chimeric B cells are responsive to TNP-Ficoll without any further requirement for MHC-restricted cell interactions.

Chimeric B Cells Are MHC Restricted in the Presence of Conventional T_H Cells. The above experiments demonstrate that soluble products of monoclonal T cells derived either by Con A stimulation or specific antigen stimulation can activate TNP-Ficoll-responsive B cells in the absence of demonstrable MHC-restricted interactions with either T cells or accessory cells. However, none of these T cell supernatants were derived from T cells stimulated with TNP-Ficoll. Indeed, it was conceivable that TNP-Ficoll does not stimulate T cells to secrete soluble factors that are capable of directly activating TNP-Ficoll-responsive B cells. Consequently, the activation of TNP-Ficoll-responsive B cells by TNP-Ficoll-responsive T_H cells might still require B cell recognition of accessory cell MHC determinants. To test this hypothesis, T cell-dependent responses to TNP-Ficoll were studied in experiments in which helper activity was provided by unprimed T cell populations and in which B cells were derived from MHC-restricted allogeneic chimeras. When nylon-nonadherent T cells from normal "unrestricted" (B10 × B10.BR)F_1 mice were added in titrated numbers to cultures of T-depleted B10 → B10.BR or B10.BR → B10 spleen cells, no substantial responses were generated (Fig. 6). Thus, any MHC restrictions involved in the activation of chimeric B cells were not overcome by the addition of T_H cells, although these T cells were fully competent to help both normal B10 and normal B10.BR B cells for the same responses (data not shown). However, when these same T_H cells were added to a mixture of the same T-depleted B10 → B10.BR and B10.BR → B10 spleen cells, a TD response to TNP-Ficoll was induced, thereby demonstrating that the chimeric B cells were not intrinsically unable to cooperate with normal F_1 T_H population (Fig. 6). Thus, these results suggest that the chimeric B cells are MHC restricted in these responses, and that even when T_H cell restrictions are satisfied, chimeric B cells must still interact with accessory cells of the chimeric host haplotype that are present in the T-depleted spleen cell population of the reciprocal chimera.

To further assess the existence of MHC restriction requirements for the activation

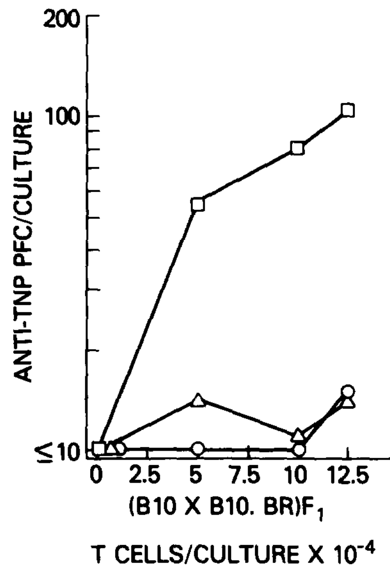

FIG. 6. Non-T cells are MHC restricted in TD responses to TNP-Ficoll. Titrated numbers of nylon-nonadherent (B10 × B10.BR)F1 spleen (T) cells were added to 4 × 10^5 anti-Thy-1.2 plus C-treated B10 → B10.BR (○) or B10.BR → B10 (△) spleen B plus accessory cells, or to a mixture of 2 × 10^5 B10 → B10.BR plus 2 × 10^5 B10.BR → B10 B plus accessory cells (□), and cultures were assayed for responses to 2 × 10^{-3} μg/ml TNP-Ficoll. Responses in the absence of antigen were <10 PFC/culture.

of TNP-Ficoll-responsive chimeric B cells, three-cell mixing experiments were carried out in which the requirements for T cells, B cells, and accessory cells could each be assessed. T cells of (B10 × B10.A)F1 origin were titrated into cultures of T-depleted B10.A → B10 spleen B plus accessory cells with the addition of B10 or B10.A SAC as a source of accessory cells, and responses to 2 × 10^{-3} μg/ml TNP-Ficoll were measured. In the absence of added SAC or in the presence of added donor-type B10.A SAC, F1 T cells did not allow responses by chimeric B cells (Fig. 7). However, in the presence of host-type B10 SAC, F1 T cells did cooperate with B10.A → B10 B cells for responses to TNP-Ficoll. Thus, even in the presence of competent "unrestricted" F1 T_H cells, chimeric B cells were MHC restricted in their requirement for accessory cells of host *H-2* haplotype in these responses.

Soluble T Cell Factors Overcome the Requirement for Self-MHC Recognition by TNP-Ficoll-responsive B Cells. Finally, to assess directly the self-recognition requirements of TNP-Ficoll-responsive B cells and the effects of soluble T cell factors on these recognition requirements, a series of co-culture experiments was performed. Unfractionated [B10.A × B10.A(2R)]F1 spleen cells were co-cultured with unfractionated B10.A → B10 chimeric spleen cells and stimulated with TNP-Ficoll. The two spleen populations were identically $H\text{-}2^a$ except that (*a*) the [B10.A × B10.A(2R)]F1 spleen cells were heterozygous $H\text{-}2D^{d/b}$, and (*b*) the B10.A → B10 chimeric spleen cells had differentiated in an $H\text{-}2^b$ environment. Upon stimulation of the co-cultures with TNP-Ficoll, the activation requirements of the normal and chimeric B cells could be compared in the presence of the same T cell and accessory cell populations. Thus, any differences observed in the activation of these two B cell populations in co-culture could be

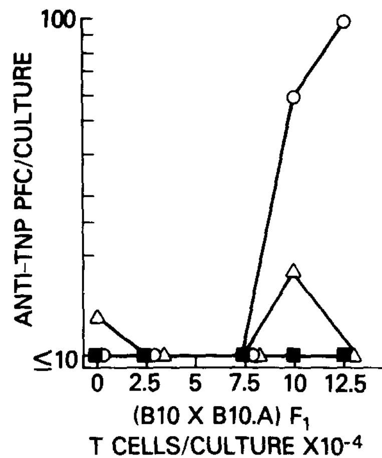

FIG. 7. Chimeric B cells are MHC restricted in their recognition of accessory cells. Titrated numbers of nylon-nonadherent (B10 × B10.A)F_1 spleen (T) cells were added to 4 × 10^5 anti-Thy-1.2 plus C-treated B10.A → B10 spleen B plus accessory cells in the presence of no added SAC (■), or in the presence of 4 × 10^4 B10 (○) or B10.A (△) SAC, and cultures were assayed for responses to 10^{-3} μg/ml TNP-Ficoll. Responses in the absence of antigen were <10 PFC/culture.

TABLE IV

Hybridoma T Cell Supernatant Overcomes the Requirement for MHC Restriction in Activation of Chimeric B Cells by TNP-Ficoll

Group	Spleen cells cultured	IL-2	Antigen	TNP-specific PFC* C	TNP-specific PFC* Anti-D^b plus C	Percent response remaining
A	B10.A	−	TNP-Ficoll	915	836	91
B	(B10.A × 2R)F_1	−	TNP-Ficoll	800	19	2
C	B10.A → B10 + (B10.A × 2R)F_1	−	TNP-Ficoll	579	21	4
D	B10.A → B10 + (B10.A × 2R)F_1	+	TNP-Ficoll	1,560	633	41

* 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, with or without a 1:2 dilution of FS6 supernatant as a source of IL-2. At the end of this culture period, cells were harvested, 10^7 cells of each group were treated with C alone or with anti-D^b antiserum plus C, and the remaining cells were assayed for anti-TNP PFC.

attributed directly to differences in B cell activation requirements and not solely to differences between normal and chimeric T cells or accessory cells. It was observed that co-cultures did respond to TNP-Ficoll (Table IV, group C); however, it can be seen that all the PFC generated were sensitive to treatment with anti-D^b plus C and hence were exclusively derived from the normal [B10.A × B10.A(2R)]F_1 B cell population (Table IV, group C). In other words, the chimeric B10.A → B10 B cells had not been triggered in co-cultures, which is consistent with the fact that the co-cultures did not contain any of the host-type H-2^b accessory cells required for the activation of these chimeric B cells. However, when soluble supernatant from the T cell hybridoma FS6 was added to spleen cell co-cultures responding to TNP-Ficoll, 41% of the PFC were insensitive to treatment with anti-D^b plus C, which demonstrates that the FS6 supernatant activated both the [B10.A × B10.A(2R)]F_1 B cells and the B10.A → B10 chimeric B cells (Table IV, group D). Thus, this experiment directly demonstrated that (*a*) TNP-Ficoll-responsive B cells that had matured in an allogeneic

differentiation environment are not activated in the presence of syngeneic T_H cells and accessory cells even though TNP-responsive normal B cells present in the same cultures are activated, and (*b*) soluble T cell factors, unlike T_H cells themselves, activate TNP-Ficoll-responsive chimeric B cells in the absence of chimeric host-type accessory cells.

Taken together, the experiments presented in this report demonstrate that soluble T cell factors elicited from monoclonal T cell populations with either Con A or specific polypeptide antigens do activate TNP-Ficoll-responsive B cells directly without any requirement for further cell interactions, whereas activation of TNP-Ficoll-responsive B cells by TNP-Ficoll-reactive T_H cells requires that both B cells and T_H cells recognize the MHC determinants expressed by TNP-Ficoll-presenting accessory cells.

Discussion

It has recently been demonstrated (12–15) that B cells, as well as T_H cells, can express requirements for recognition of self-MHC determinants expressed by the accessory cells with which they interact. In particular, it has recently been shown (15) that B cells from long-term radiation bone marrow chimeras express a requirement for MHC-restricted self-recognition in TI responses to high concentrations of TNP-Ficoll. These studies did not determine, however, the effects that T cells or soluble T cell products might exert on this B cell function. Thus, it remained undetermined whether interacting T_H cells and B cells could each express requirements for recognition of self-MHC determinants on accessory cells in a TD response. Because the simultaneous expression of such recognition requirements by both B cells and T_H cells would constitute a novel cell interaction pathway, the present study was carried out to assess the cell interaction requirements of T_H cells and B cells in the highly TD primary antibody response to low concentrations of TNP-Ficoll. It was first shown that T_H cell function under these conditions is MHC restricted, requiring T cell recognition of MHC products expressed by accessory cells but not by B cells. It was also found that the soluble products secreted by cloned T cells stimulated by Con A or by specific polypeptide antigen permitted direct B cell activation by TNP-Ficoll without MHC restriction and in fact without demonstrable requirements for either T cells or accessory cells. However, in the absence of these soluble factors and in the presence of MHC-restricted T_H cells, it was demonstrated that activation of TNP-Ficoll-responsive chimeric B cells still required B cell recognition of accessory cell MHC determinants.

These findings thus demonstrate the existence of two distinct requirements for MHC-restricted recognition in TD responses to TNP-Ficoll. T_H cells are restricted in their recognition of MHC determinants expressed on accessory cells, but show no similar requirement for recognition of B cell MHC products. These results are similar to those previously described for T_H cell-dependent activation of Lyb-5^+ B cells in responses to the conventional TD antigens TNP-KLH and SRBC (16, 21, 25). In addition, the present report has demonstrated a requirement for MHC-restricted recognition of accessory cells by responding chimeric B cells. A novel cell interaction model that is suggested by these findings is that direct and MHC-restricted interaction occurs between T cells and accessory cells as well as between B cells and accessory cells, but that there is no requirement for direct interaction between T and B cells in

this activation pathway (Fig. 8A). It is not clear from the present experiments whether MHC-restricted T_H cells and MHC-restricted B cells cooperating in the TNP-Ficoll response are required to recognize MHC determinants on the same accessory cell or whether these T and B cells must recognize the identical MHC determinants expressed on these accessory cells.

The role of soluble T cell products as mediators in responses to TNP-Ficoll is unclear. It was demonstrated that soluble products of monoclonal T cells could directly activate B cells for these responses, bypassing any apparent requirement for the participation of either T cells or accessory cells. The conclusion that these supernatants acted directly on B cells, and not on residual T cells, was supported by the finding that, as reported by Harwell et al. (20), these same supernatants were not sufficient to reconstitute anti-SRBC responses of the same adequately T-depleted populations, whereas such anti-SRBC responses were reconstituted by heterogeneous supernatants from Con A-stimulated spleen cells. In contrast, when help for responses to TNP-Ficoll was provided by unprimed T_H cells, a requirement for MHC-restricted B cell interaction with accessory cells was consistently observed. This contrast implies that stimulation of T_H cells with TNP-Ficoll fails to generate soluble lymphokines that are (qualitatively and quantitatively) sufficient to overcome the requirement for MHC-restricted cell interaction with accessory cells in the activation of B cells. Furthermore, these observations predict that a requirement for MHC-restricted self-recognition by B cells will not be observed under conditions in which such lymphokines are induced (Fig. 8B).

It remains to be determined how this model of MHC-restricted self-recognition will apply to the activation of B cells participating in conventional TD responses to polypeptide antigens. Although the T_H cells participating in responses to TNP-Ficoll express MHC restriction requirements similar to those of the T_H cells that activate Lyb-5^+ B cells for responses to polypeptide antigens (16, 21, 25, 26), it is likely that these T_H cells in fact represent distinct populations. Thus, for example, antigen-specific T cell proliferative responses have been generated to a wide variety of polypeptide antigens, but not to the polysaccharide TNP-Ficoll. In addition, only modest priming effects have as yet been reported for antibody responses to TNP-Ficoll (24), and the antigen specificity of TNP-Ficoll-responsive T_H cells, in contrast to that of polypeptide-specific T_H cells, has not yet been established. In particular, the possibility must be considered that TNP-Ficoll may differ from conventional TD polypeptide antigens in its failure to efficiently stimulate the secretion of soluble T_H

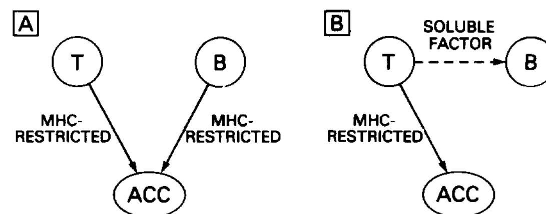

FIG. 8. (A) The T cell-dependent activation of TNP-Ficoll-responsive B cells can occur through a novel activation pathway in which T_H cells and B cells are each required to recognize self-MHC determinants expressed on accessory cells. (B) Soluble T cell factors can directly activate TNP-Ficoll-responsive B cells by an alternative pathway that bypasses cell interaction requirements and thus overcomes any requirement for B cell recognition of self-MHC products.

cell products capable of directly activating B cells. The ability of polypeptide antigens to stimulate such T_H cell factors might well favor the direct activation of B cells in these responses (Fig. 8B), thereby obscuring alternative pathways in which B cells might express a requirement for recognition of self-MHC products expressed on accessory cells (Fig. 8A).

In conclusion, the present studies have investigated the effect of T cells and soluble T cell products on the activation requirements of TNP-Ficoll-responsive B lymphocytes. It was observed that the T_H cells required for responses to limiting concentrations of TNP-Ficoll were MHC restricted in their interactions with accessory cells. In addition, chimeric B cells responding in the presence of these T_H cells also expressed a requirement for MHC-restricted self-recognition in their interaction with accessory cells, although this requirement could be bypassed in the presence of soluble T cell factors. Thus, the present studies demonstrate that requirements for self-recognition of accessory cell MHC determinants by TNP-Ficoll-responsive B cells can only be observed under conditions in which a B cell-accessory cell interaction occurs and can be bypassed by the presence of soluble B cell activating factors derived from T cells. In addition, the present study demonstrates the existence of a novel cell interaction pathway in which TNP-Ficoll-reactive T_H cells and B cells are each required to recognize accessory cell MHC determinants, but are not required to recognize each other.

Summary

In vitro primary antibody responses to limiting concentrations of trinitrophenyl (TNP)-Ficoll were shown to be T cell dependent, requiring the cooperation of T helper (T_H) cells, B cells, and accessory cells. Under these conditions, T_H cells derived from long-term radiation bone marrow chimeras were major histocompatibility complex (MHC) restricted in their ability to cooperate with accessory cells expressing host-type MHC determinants. The requirement for MHC-restricted self-recognition by TNP-Ficoll-reactive B cells was assessed under these T-dependent conditions. In the presence of competent T_H cells, chimeric B cells were found to be MHC restricted, cooperating only with accessory cells that expressed host-type MHC products. In contrast, the soluble products of certain monoclonal T cell lines were able to directly activate B cells in response to TNP-Ficoll, bypassing any requirement for MHC-restricted self-recognition. These findings demonstrate the existence of a novel cell interaction pathway in which B cells as well as T_H cells are each required to recognize self-MHC determinants on accessory cells, but are not required to recognize each other. They further demonstrate that the requirement for self-recognition by B cells may be bypassed in certain T-dependent activation pathways.

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