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# ANTIGEN PRESENTATION BY HAPTEN-SPECIFIC B LYMPHOCYTES

## I. Role of Surface Immunoglobulin Receptors

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Cells of the monocyte/macrophage lineage, dendritic cells, and a variety of other cell types that express class II histocompatibility (Ia) molecules on their surfaces are capable of presenting foreign protein antigens to and stimulating Ia-restricted inducer (helper) T lymphocytes (1-3). After the demonstration that B cells also bear Ia antigens (4) and that at least some interactions between antigen-specific helper T cells and B lymphocytes are Ia restricted (5), many investigators have attempted to determine whether B lymphocytes can also function as antigen-presenting cells. Among the earliest studies to demonstrate that B cells can, in fact, present antigens to T cells were the experiments showing that macrophage-depleted murine B cells incubated with protein antigens could stimulate immune T lymphocytes to proliferate in an Ia-restricted fashion (3). More convincing evidence for the role of B cells in presenting antigen came from studies of Chesnut and Grey (6), who showed that macrophage-depleted splenocytes cultured with rabbit anti-mouse immunoglobulin (Ig), but not rabbit IgG lacking anti-Ig activity, could stimulate rabbit  $\gamma$ -globulin-primed T cells to proliferate in vitro (6). This result not only established the ability of B lymphocytes to present protein antigens to T cells but also indicated that their surface Ig receptors played an important role in this phenomenon. Subsequently, it has been shown that resting and mitogen-activated normal B lymphocytes as well as a variety of Ia-positive B cell-derived tumors and cell lines are capable of presenting foreign proteins to Ia-restricted, antigen-reactive T lymphocytes (7-12). In all these situations, high concentrations of antigens are required and surface Ig receptors play no role. In fact, the amounts of antigens required for such presentation are comparable to those observed with other accessory cells (1, 2), but in vast excess of the concentrations required for T cell-dependent, antigen-specific, major histocompatibility-restricted activation of primed B lymphocytes (13). Thus, although cloned B cell tumors and normal B lymphocytes are valuable for analyzing the cellular and molecular events in antigen presentation (e.g., 14, 15), the relevance of such studies to physiologic antigen-specific T-B interactions involving cognate recognition of linked antigenic determinants is unclear.

In an attempt to develop systems for analyzing the interactions between antigen-specific T and B lymphocytes, we have investigated the ability of B cells

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enriched for expression of surface receptors for a defined hapten to present hapten-protein to carrier-specific T cells. In the initial experiments, primed murine B lymphocytes specific for 2,4,6-trinitrophenyl (TNP)<sup>1</sup> were used to present a TNP-modified, Ir gene-controlled synthetic terpolymer of glutamic acid, lysine, and phenylalanine (GL $\phi$ ) to a GL $\phi$ -reactive T cell hybridoma. Such cloned hybridoma cells, which secrete interleukin 2 (IL-2) upon interaction with the relevant nominal antigen and Ia determinant, have been extensively characterized and are widely used to analyze the requirements for T cell activation (16–17). Our experiments demonstrate that hapten-specific B lymphocytes are highly efficient at presenting hapten-protein to T cells, and that hapten-binding surface Ig molecules are critically involved in this effective form of T-B interaction. Moreover, in this system it appears that the major role of surface Ig is to concentrate or “focus” antigen on to relevant B cells such that very low concentrations of antigen are able to maximally stimulate the responding T lymphocytes. The results also raise the possibility that antigen-binding B cells may serve an important role as antigen-presenting cells in physiologic immune responses.

### Materials and Methods

*Mice.* BALB/c mice, ages 6–12 wk, were purchased from Charles River Breeding Laboratories, Inc., Kingston, NY or Cumberland Farms, Clinton, TN. Animals used in this study were maintained in accordance with the guidelines of the Committee on Animals of the Harvard Medical School and those prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 78-23, revised 1978).

*Antigens and Antibodies.* Hapten conjugates of GL $\phi$ , keyhole limpet hemocyanin (KLH), human serum albumin (HSA), bovine  $\gamma$ -globulin (BGG), and lipopolysaccharide (LPS) were prepared by incubating the carriers with 2,4,6-trinitrobenzene sulfonic acid (TNBS) at pH 9–9.5 for 4–6 h, and removing unbound TNBS by dialysis or gel filtration.

Rabbit anti-mouse Ig was prepared from a hyperimmune rabbit anti-mouse F(ab')<sub>2</sub> serum by affinity chromatography on mouse  $\gamma$ -globulin-coupled Sepharose 4B, digested with pepsin, and the F(ab')<sub>2</sub> fragment purified by passage over protein A-Sepharose. A control normal rabbit F(ab')<sub>2</sub> was prepared from rabbit serum absorbed with mouse  $\gamma$ -globulin. The following anti-TNP mouse sera were used: hyperimmune BALB/c anti-TNP-KLH serum and sera from mice immunized 2–6 wk previously with TNP-KLH in complete Freund's adjuvant and used as donors for B cells (see below). All these sera were heat inactivated and shown to have TNP binding activity at dilutions >1:10<sup>6</sup> in a sensitive, solid phase radioimmunoassay using TNP-bovine albumin-coated microtiter plates. In addition, two monoclonal anti-TNP antibodies were used: an IgG1, $\kappa$  (termed HDP<sub>1</sub>; provided by Dr. H. Urnovitz and Dr. R. Lynch, University of Iowa) and an IgM, $\kappa$  (termed T2.8), prepared by fusion of TNP-Ficoll-immunized BALB/c spleen cells. Both of these showed anti-TNP activity at titers >1:10<sup>2</sup> in the solid phase radioimmunoassay. The following monoclonal anti-Ia antibodies were used in blocking experiments at a 1:8 dilution of hybridoma culture supernatant: MKD6 (18) (anti-I-A<sup>d</sup>) and 14.4.4s (19) (anti-I-E<sup>d</sup>).

*Hapten-specific B Lymphocytes.* Spleen cells were obtained from BALB/c mice immunized 6 d previously with 0.5  $\mu$ g of TNP-LPS in aqueous solution intraperitoneally (i.p.)

<sup>1</sup> *Abbreviations used in this paper:* BGG, bovine  $\gamma$ -globulin; GAT, random terpolymer of glutamic acid<sup>60</sup>, alanine<sup>30</sup>, and tyrosine<sup>10</sup>; GL $\phi$ , random terpolymer of glutamic acid<sup>56</sup>, lysine<sup>35</sup>, and phenylalanine<sup>9</sup>; HSA, human serum albumin; IL-2, interleukin 2; KLH, keyhole limpet hemocyanin; LPS, *E. coli* lipopolysaccharide; MEM, minimum essential medium with 5% heat-inactivated fetal calf serum; MHC, major histocompatibility complex; TNBS, 2,4,6-trinitrobenzene sulfonic acid; TNP, 2,4,6-trinitrophenyl.

or 2–6 wk previously with 100  $\mu\text{g}$  of TNP-KLH in complete Freund's adjuvant i.p. Hapten-binding B cells were purified by a modification of the technique of Haas and Layton (20). Erythrocyte-free spleen cells were suspended in minimum essential medium with 5% heat-inactivated fetal calf serum (MEM) and incubated on 100-mm plastic culture dishes coated with TNP-gelatin, at a ratio of  $100\text{--}150 \times 10^6$  cells in 5 ml per dish. Dishes were rocked gently at 4°C for 60 min, washed five times with ice-cold MEM, and bound cells eluted by adding 5 ml of MEM warmed to 37°C. Cells were treated with 100 U/ml of collagenase (Worthington Biochemical Corp., Freehold, NJ) for 15 min at 37°C to remove bound TNP-gelatin, washed three times, and viable cell recovery determined by trypan blue dye exclusion. In our hands, 0.5–1.5% of input splenocytes were recovered from TNP-gelatin dishes; 50% of these cells expressed surface Ig by immunofluorescence and 20–40% have detectable TNP receptors as determined by rosette assays with TNP-coupled sheep erythrocytes. Both these assays may underestimate the number of hapten-specific cells because of receptor modulation during the purification procedure. After 4 d stimulation with LPS in vitro, the TNP-gelatin-enriched cells show a 50–100-fold enrichment of anti-TNP antibody-secreting cell precursors (on a per cell basis) compared with the initial, unpurified splenocyte population. The cells eluted from hapten-gelatin are referred to as hapten-specific B lymphocytes in the following sections.

*T Cell Hybridomas.* RF21.21.9 was obtained from the fusion of BALB/c GL $\phi$ -immune, antigen-restimulated, proliferating T cells to the AKR thymic lymphoma, BW 5147, as previously described (17). It produces IL-2 when stimulated with GL $\phi$  in association with I-E<sup>d</sup>. This response is exquisitely antigen specific and H-2 restricted (K. L. Rock and B. Benacerraf, manuscript in preparation). RF7.24.3 is a BALB/c, I-A<sup>d</sup> + GAT-specific, IL-2-producing T cell hybridoma that was similarly derived (21).

*Cell Culture and Assay Conditions.* Culture media was RPMI 1640 (M. A. Bioproducts, Walkersville, MD) supplemented as previously described (21).  $5\text{--}7.5 \times 10^4$  T cell hybridomas were cultured in 200- $\mu\text{l}$  flat-bottom microcultures with or without accessory cells, in the presence or absence of antigen and in some cases antibody. The source of accessory cells was either hapten-specific, unirradiated B cells, irradiated (1,600 rad) or unirradiated whole spleen cells, or the in vitro passaged, cloned B lymphoblastoid tumor, A20.2J (16). The precise numbers of accessory cells and the concentration of antigen or antibody are detailed in the respective experimental protocols. After 18–24 h incubation at 37°C, 100  $\mu\text{l}$  of culture supernatant was removed, exposed to 8,000 rad gamma irradiation, and assayed for IL-2 content. IL-2 was measured by quantitating the incorporation of [<sup>3</sup>H]-thymidine into DNA of an IL-2-dependent T cell line, HT-2, in response to this lymphokine (18, 21). Data are expressed as the arithmetic mean counts per minute of duplicate cultures.

## Results

*Presentation of Hapten-modified Antigen by Hapten-specific B Cells.* In the first series of experiments we compared the ability of TNP-specific BALB/c (H-2<sup>d</sup>) B lymphocytes to present GL $\phi$  and TNP-modified GL $\phi$  to the GL $\phi$ -specific, I-E<sup>d</sup>-restricted T cell hybridoma, RF21.21.9. As shown in Fig. 1, such hapten-specific B lymphocytes were capable of presenting the TNP-protein at a <10-fold lower concentration than the unmodified protein. A more detailed analysis of the antigen concentrations and presenting cell numbers required to activate the T cell hybridoma is shown in Figs. 2 and 3. Hapten-specific B cells presented TNP-GL $\phi$  at concentrations as low as 0.1  $\mu\text{g}/\text{ml}$ , and as few as  $7 \times 10^3$  cells were effective at presenting haptenated protein. It is noteworthy that with  $2 \times 10^5$  TNP-specific B cells, TNP-GL $\phi$  at 0.1  $\mu\text{g}/\text{ml}$ , which is the lowest concentration we have tested, produced >70% of the maximal T cell response (Fig. 2). In contrast, the same B cells presented unmodified GL $\phi$  only at high concentrations (100  $\mu\text{g}/\text{ml}$ ) and relatively high cell numbers ( $2 \times 10^5$ ). Thus, hapten-specific B

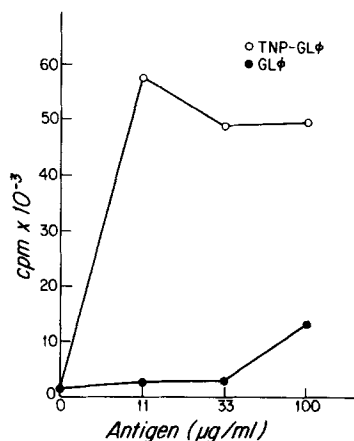


FIGURE 1. Comparison of hapten-specific B cell antigen presentation of native and hapten-conjugated GL $\phi$ . Microcultures were prepared with  $5 \times 10^4$  RF21.21.9 T cell hybrid and  $6 \times 10^5$  TNP-binding B cells in the presence or absence of indicated amount of antigen (O, TNP-GL $\phi$ ; ●, GL $\phi$ ) in 200  $\mu$ l. After 18 h incubation, 100  $\mu$ l of culture supernatant was removed, irradiated, and assayed for IL-2 content. Control experiments have shown no detectable IL-2 in cultures of B cells plus antigen, in the absence of specific T cell hybrids (data not shown). Hapten-specific B cells were isolated from TNP-LPS-primed mice.

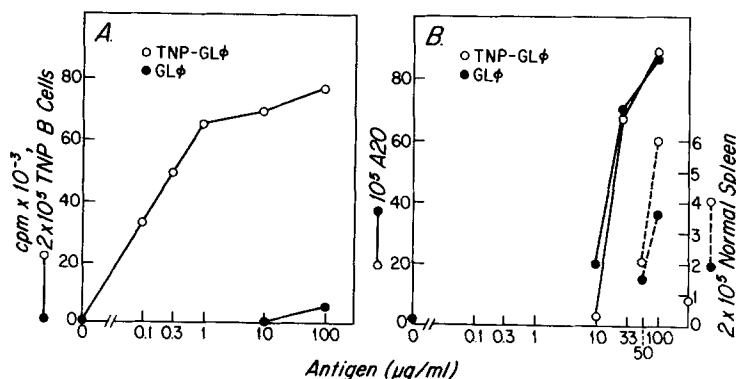


FIGURE 2. Comparison of hapten-conjugated antigen presentation by antigen-specific and naive B cells. Microcultures were prepared as described in Fig. 1 except that the source of antigen-presenting cells was varied: (A)  $2 \times 10^5$  TNP-binding B cells from TNP-LPS-primed mice; (B)  $10^5$  A20 B-lymphoblastoid cells (—) or  $2 \times 10^5$  unirradiated, naive splenocytes (---). Both A and B are from the same experiment.

cells presented the haptenated antigen >1,000-fold more efficiently than the unmodified antigen. This effect was hapten specific, since GL $\phi$  modified with other haptens, such as fluorescein, was presented by TNP-specific B cells like the unhaptenated protein (data not shown). Moreover, TNP-GL $\phi$  was not intrinsically more immunogenic than GL $\phi$ , as both A20.2J B lymphoma cells and naive, unpurified splenocytes (Fig. 2) showed no difference in their ability to present the two antigens. Both of these accessory cell populations required high concentrations of antigen (10–100  $\mu$ g/ml), which was precisely the concentration of unhaptenated antigen required by hapten-specific B cells (Figs. 1 and 2). Controls done with these and subsequent experiments showed that in the absence of either

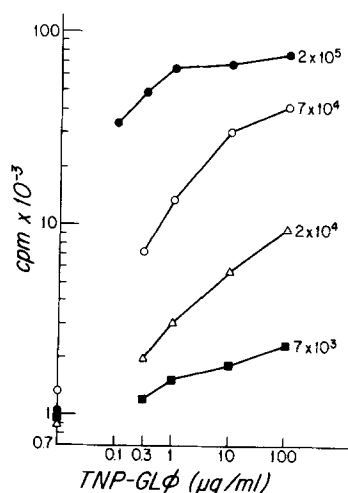


FIGURE 3. Titration of the number of TNP-specific B cells required for antigen presentation. Microcultures were prepared as described in Fig. 1, with the indicated number of TNP-binding B cells and the presence or absence of the indicated concentration of TNP-GL $\phi$ . This data was obtained from the same experiment illustrated in Fig. 2.

antigens or presenting cells, the T cell hybridoma was not stimulated to produce IL-2. Furthermore, the hapten-specific B cells alone did not produce IL-2 upon challenge with TNP-GL $\phi$  or GL $\phi$ , as expected (data not shown). Identical results were obtained with B cells from TNP-LPS- and TNP-KLH-primed mice.

*I-E Restriction of Antigen Presentation by B Lymphocytes.* Monoclonal antibody blocking experiments were done to determine the major histocompatibility complex (MHC) restriction of antigen presentation by hapten-specific B cells. As shown in Table I, stimulation of the I-E<sup>d</sup>-restricted T cell hybridoma by BALB/c TNP-specific B cells and TNP-GL $\phi$  was markedly inhibited by monoclonal anti-I-E<sup>d</sup> relative to anti-I-A<sup>d</sup> antibody. This effect was specific, since both antibodies bind to the presenting cells, and the reciprocal pattern of inhibition was observed with I-A<sup>d</sup>-restricted T cell hybridomas. The same pattern of inhibition was seen with either TNP-specific B lymphocytes or A20.2J cells and high concentrations of unmodified GL $\phi$  (data not shown).

*Role of Hapten-binding Ig Receptors in Antigen Presentation.* The above experiments indicated that cell populations enriched for hapten-specific B lymphocytes were also markedly and selectively enriched for cells capable of presenting hapten-modified proteins to Ia-restricted T cells. To establish a role for surface Ig in this phenomenon, attempts were made to block antigen presentation with ligands that would be expected to bind to receptors for the hapten. As shown in Table II, preincubation of TNP-specific B cells with affinity-purified rabbit anti-mouse Ig completely blocked the ability of these cells to present TNP-GL $\phi$  to the T cell hybridoma, RF21.21.9, and TNP-conjugated HSA or BGG inhibited the presentation of TNP-GL $\phi$  by 80–90%. Unhaptened proteins (Table II) or rabbit Ig (data not shown) had no effect. This inhibition was highly specific, since the anti-Ig and TNP-proteins did not interfere with the ability of the same TNP-specific B cells to present GL $\phi$  at high concentrations, despite the response being

TABLE I  
Effect of Monoclonal  $\alpha$ -Ia Antibody on Antigen Presentation of TNP-GL $\phi$

Hybrid	$2 \times 10^5$ TNP-B cells	Antigen $\mu\text{g/ml}$	Monoclonal antibody	cpm
RF21.21.9	+	—	—	2,981
	+	10 GL $\phi$	—	2,564
	+	1 TNP-GL $\phi$	—	35,289
	+	1 TNP-GL $\phi$	I-E <sup>d</sup>	4,911
	+	1 TNP-GL $\phi$	I-A <sup>d</sup>	15,863
RF7.24.3	+	—	—	2,722
	+	100 GAT	—	23,964
	+	100 GAT	I-E <sup>d</sup>	16,262
	+	100 GAT	I-A <sup>d</sup>	3,588

Microcultures were prepared as described in Fig. 1, except  $2 \times 10^5$  TNP-binding B cells were used and a 1:8 dilution of monoclonal antibody-containing culture supernatant was added where indicated. RF7.24.3 is a GAT + I-A<sup>d</sup>-specific T cell hybrid included as a reciprocal control. TNP-specific cells were obtained from TNP-LPS-primed mice.  $\alpha$ -I-E<sup>d</sup>, 14.4.4.S;  $\alpha$ -I-A<sup>d</sup>, MKD6.

substantially weaker. Moreover, none of the inhibitors affected the ability of non-TNP-binding A20.2J B cells to present the same antigen, TNP-GL $\phi$  (Table II). These results demonstrate that the augmented presentation of TNP-protein by TNP-binding B cells is specific for the hapten and not the carrier, and directly implicate B cell Ig in this highly efficient presentation of haptened antigen.

*Function of Ig Receptors in Antigen Presentation.* There are several, not mutually exclusive, reasons why hapten-binding B lymphocytes might be particularly efficient at presenting haptened antigen to Ia-restricted T cells. The simplest is that Ig receptors serve to focus antigen, and perhaps direct its pathway of endocytosis and processing. Alternatively, the binding of antigen to surface receptors may deliver a signal(s) that activates target B lymphocytes to become more efficient at presenting antigen; such activation events may include enhanced expression of Ia determinants (22–24). To test this possibility, TNP-specific B cells with and without TNP-GL $\phi$  were compared for their ability to stimulate a T cell hybridoma specific for an unrelated, unmodified protein, GAT (a terpolymer of glutamic acid<sup>66</sup>, alanine<sup>30</sup>, and tyrosine<sup>10</sup>), or to stimulate an alloreactive T cell hybridoma. As shown in Table III, the continuous presence of TNP-GL $\phi$  at a concentration at which it was effectively presented did not alter the ability of TNP-specific B cells to present GAT to a GAT + I-A<sup>d</sup>-specific T cell hybridoma. Similarly, TNP-GL $\phi$  did not make the TNP-specific B cells more efficient stimulators of an allogeneic I-E<sup>d</sup>-reactive T cell hybridoma. This is particularly relevant since I-E<sup>d</sup> is the restriction element of the GL $\phi$ -reactive hybrid, RF21.21.9. Finally, the presence of TNP-conjugated HSA or BGG did not activate the hapten-specific B cells to more efficiently present unmodified GL $\phi$  to RF21.21.9, as they would TNP-GL $\phi$  (Table II). Therefore, the interaction of TNP-conjugated antigens with the B cells fails to markedly enhance their ability to present unhaptened antigens in a noncognate manner.

TABLE II  
*Hapten- and Immunoglobulin-specific Blocking of TNP-GL $\phi$  Presentation by Specific B Cells*

Hybrid	Antigen-presenting cells	Antigen ( $\mu\text{g/ml}$ )	Inhibiting antigen/Ab ( $\mu\text{g/ml}$ )	cpm	Percent inhibition	
RF21.21.9	$2 \times 10^5$ TNP-B	—	—	1,051		
		0.3 TNP-GL $\phi$	—	—	49,206	
	50 RAMG F(ab') <sub>2</sub>		1,257	100		
	100 TNP-HSA		7,428	88		
	100 HSA		52,768	0		
	100 TNP-BGG		8,806	84		
	100 BGG		48,781	1		
	$2 \times 10^5$ TNP-B	100 GL $\phi$	—	—	5,345	
			50 RAMG F(ab') <sub>2</sub>	9,623	0	
		100 TNP-HSA	13,054	0		
		100 HSA	4,134	28		
		100 TNP-BGG	5,356	0		
		100 BGG	4,492	20		
	$2 \times 10^5$ TNP-B	—	—	50 RAMG F(ab') <sub>2</sub>	1,412	
		—	—	100 TNP-HSA	2,051	
—		—	100 HSA	1,305		
—		—	100 TNP-BGG	2,198		
—		—	100 BGG	1,254		
RF21.21.9	$10^5$ A20	—	—	1,801		
		25 TNP-GL $\phi$	—	—	68,831	
	50 RAMG F(ab') <sub>2</sub>		66,470	0		
	100 TNP-HSA		47,534	31		
	100 HSA		58,948	14		
	100 TNP-BGG	68,190	0			
100 BGG	77,160	0				

Microcultures were prepared as described in Table I except for the addition of the indicated amount of rabbit anti-mouse Ig (RAMG) F(ab')<sub>2</sub> or competing antigen. These potential inhibitors were incubated with B cells at 37°C for 60 min before the addition of hybrids, and were continuously present throughout the remainder of the culture. Data from one representative experiment out of two are shown. In the second experiment, RAMG F(ab')<sub>2</sub> inhibited presentation of TNP-GL $\phi$  by TNP-specific B cells by 100%, while normal rabbit F(ab')<sub>2</sub> had no effects.

It is possible that TNP-specific, immunized B lymphocytes secrete small amounts of antihapten antibody that complexes with and augments the presentation of hapten-protein. To test this possibility, A20.2J cells were incubated with TNP-GL $\phi$  at a suboptimal concentration, with and without a wide range of dilutions of four different anti-TNP antibodies (two sera and two monoclonals both IgG and IgM), and the IL-2 response of the RF21.21.9 hybridoma measured. None of the antibodies tested altered the presentation of TNP-GL $\phi$  under these conditions (Table IV).

#### Discussion

The experiments reported in this paper were designed to examine the ability of antigen-binding B lymphocytes to present the specific antigen to Ia-restricted



TABLE III  
*Haptenated Antigen Does Not Augment Antigen or MHC Molecule Presentation in a Noncognate Manner*

Hybrid	Hybrid specificity	TNP-B cells ( $\times 10^5$ )	Antigen ( $\mu\text{g}/\text{ml}$ )	TNP-GL $\phi$ (1 $\mu\text{g}/\text{ml}$ )	cpm
RF7.24.3	GAT + I-A <sup>d</sup>	2	—	—	2,722
		2	10 GAT	—	18,549
		2	1 GAT	—	8,047
		2	1 GAT	+	5,132
RF26.12	I-E <sup>d</sup> (Allo)	—	—	—	3,486
		1	—	—	33,840
		0.5	—	—	31,074
		0.25	—	—	15,042
		0.125	—	—	8,064
		0.075	—	—	5,502
		1	—	+	36,233
		0.5	—	+	26,184
		0.25	—	+	23,129
		0.125	—	+	10,565
0.075	—	+	6,313		

Microcultures were prepared as described in Table I except using the RF7.24.3 and RF26.12 hybrids and the corresponding antigen to RF7.24.3, GAT. Data was obtained for the same experiment illustrated in Table I, demonstrating that 1  $\mu\text{g}/\text{ml}$  of TNP-GL $\phi$  was presented strongly in a cognate manner.

TABLE IV  
*Anti-TNP Antibody Does Not Enhance the Presentation of TNP-GL $\phi$*

Hybrid	Antigen-presenting cells	Antigen ( $\mu\text{g}/\text{ml}$ )	Antibody*	cpm
RF21.21.9	TNP-B cell	—	—	312
		10 GL $\phi$	—	268
		1 TNP-GL $\phi$	—	9,299
A20	A20	—	—	347
		100 TNP-GL $\phi$	—	13,520
		33 TNP-GL $\phi$	—	1,782
		10 TNP-GL $\phi$	—	343
		1 TNP-GL $\phi$	—	1,046
A20	A20	1 TNP-GL $\phi$	1:10 <sup>-4</sup> hyperimmune serum	816
			1:10 <sup>-5</sup> hyperimmune serum	669
			1:10 <sup>-2</sup> donor serum	638
			1:10 <sup>-3</sup> donor serum	693
			1:4 T2.8 MAb	964
			1:8 T2.8 MAb	871
1:4 HDP <sub>1</sub> MAb	627			
1:8 HDP <sub>1</sub> MAb	633			

Microcultures were prepared as described in Table I except that either  $2.5 \times 10^5$  TNP-B cells from TNP-KLH-primed mice or  $5 \times 10^4$  A20 cells were used and the indicated source of anti-TNP antibody was added.

\* Donor serum was serum obtained from the immune B cell donors. MAb, monoclonal antibody.

T cells. Using a model system in which TNP-primed B lymphocytes, enriched for hapten-binding cells, were used to present a TNP-modified protein, GL $\phi$ , to a GL $\phi$ -reactive, I-E<sup>d</sup>-restricted T cell hybridoma, we have observed that such B cells are remarkably efficient antigen-presenting cells. Hapten-specific B lymphocytes were capable of activating the T cell hybridoma in the presence of soluble hapten-protein at 100 ng/ml, which is a lower antigen concentration for effective presentation to T cells than has been described in any *in vitro* system, to date. Moreover, as few as  $7 \times 10^3$  hapten-enriched cells were sufficient to stimulate the relevant T cell hybridoma. Considering the low numbers of hapten-specific B lymphocytes that are effective at presenting antigen, it is likely that these cells alone are sufficient for stimulating the T cell response. Recent experiments (work in progress) using transformed, antigen-specific B cell clones support this interpretation. However, it should be pointed out that the question of whether other cell types present in the hapten-enriched population also play a role in activating T lymphocytes has not been addressed by our experiments.

Several lines of evidence demonstrated a central role of surface Ig in the presentation of hapten-protein by hapten-specific B cells. First, TNP-specific B cells presented low concentrations of TNP-GL $\phi$  but not unmodified GL $\phi$  (Fig. 2) or fluorescein-conjugated GL $\phi$  (not shown) to the same GL $\phi$ -reactive T cell hybridoma. Second, the presentation of TNP-GL $\phi$  by the B cells was specifically inhibited by anti-Ig antibody and by other TNP-modified proteins (Table II). In this respect, our observations are fundamentally different from most results of antigen presentation by B lymphocytes. Thus, resting, activated, and neoplastic B cells are capable of presenting protein antigens to Ia-restricted T cells (6-12), but in all these cases Ig receptors play no role. The only situation in which a function for B cell Ig in antigen presentation has been demonstrated is the study of Chesnut and Grey (6) showing that rabbit anti-Ig is presented by partially purified B cell populations under conditions where normal rabbit Ig is not. Even in these experiments, however, high concentrations of protein antigen were required, comparable to the concentrations at which effective presentation is observed with other accessory cells such as macrophages and dendritic cells.

It has been postulated that Ig receptors on B lymphocytes may serve at least two functions relevant to the ability of these cells to present antigens to T cells. The most obvious is that receptors focus antigen on the cell surface and lead to a sequence of endocytosis and processing that culminates in the expression of antigenic determinants in association with Ia molecules. In addition, the antigen-receptor interaction may activate B cells to a state at which they are efficient at antigen presentation; such activation may include enhanced expression of Ia, more efficient antigen processing, and other, as yet undefined, changes in the physiologic properties of the cells (22-24). In our experiments, TNP-GL $\phi$  did not enhance the ability of TNP-specific B cells to present an unrelated, nonhaptenated protein or to stimulate alloreactive T cell hybridomas (Table III), and the enhanced presentation always required linked or cognate recognition of hapten-carriers by carrier-specific T cell hybridomas. Moreover, there was no evidence that antibody secretion could account for the ability of these cells to present TNP-protein (Table IV). Therefore, by exclusion, it is likely that the only or major role of surface Ig in antigen presentation by B cells is to bind and

focus the specific antigen. It should be emphasized that the B cells used in the present experiments were obtained from primed animals and were, therefore, previously activated. It is certainly conceivable that in resting B cells, the activation induced by antigen-receptor interactions plays a more important or readily demonstrable role in antigen presentation. In any event, it is likely that antigen binds to surface Ig and is endocytosed and degraded by proteolytic enzymes; such a sequence of events has been demonstrated with anti-Ig antibodies (25). Thus, in general, the cellular processes leading to Ia-associated antigen presentation may be similar in B lymphocytes and in more intensively studied antigen-presenting cells, such as monocytes/macrophages and possibly dendritic cells (1, 2).

The ability of B cells to present nominal protein antigens to inducer T cells has several implications for physiologic immune responses. It may provide an accessory cell-independent mechanism for stimulating inducer cells to secrete helper factors that are necessary for clonal expansion and differentiation of B lymphocytes. The critical role of surface Ig in this phenomenon suggests that B cells are most efficient at presenting the antigen for which they express specific membrane receptors. This provides an explanation for the observation that physiologic cognate T-B collaboration occurs at concentrations of antigens that are insufficient to cause detectable activation of helper T cells in conventional *in vitro* assays for DNA synthesis in which antigen is presented by macrophages or dendritic cells. Furthermore, it would account for the finding that in most antibody responses *in vivo*, activation of bystander B cells is rarely or never observed. This may also explain, at least in part, the stringent MHC restriction of T-B interactions observed *in vivo* and in secondary antibody responses to low dose antigen challenge *in vitro* (5, 26).

Finally, our finding that B cells present antigens at concentrations approaching expected physiologic levels suggests that such cells may play a major role in presenting antigen, at least to previously activated T lymphocytes, *in vivo*. Whether, in addition to this ability to present antigen in association with Ia molecules, B cells produce secondary activation signals, e.g., interleukin 1, is not known (17). Such antigen-nonspecific cytokines may be important for the activation of naive or resting T lymphocytes. Thus, it remains to be determined whether or not B lymphocytes are also capable of initiating antigen-specific immune responses, i.e., by stimulating normal, resting inducer T cells.

### Summary

The present study examines the ability of hapten-specific murine splenic B lymphocytes to present hapten-proteins to carrier-specific T cell hybridomas. BALB/c B cells specific for 2,4,6-trinitrophenyl (TNP) were isolated from spleens of immune mice by elution from TNP-gelatin-coated dishes. Such cells presented the TNP-modified terpolymer, GL $\phi$ , at concentrations as low as 0.1  $\mu$ g/ml, to a GL $\phi$ -specific, I-E<sup>d</sup>-restricted, interleukin 2-producing T cell hybridoma. In contrast, the same B lymphocytes required 1,000-fold higher concentrations of unmodified GL $\phi$  to stimulate the same T cell hybridoma. The presentation of low concentrations of TNP-GL $\phi$  by TNP-specific B lymphocytes was significantly or completely blocked by anti-Ig antibody or TNP-proteins, indicating that

surface Ig receptors were critically involved in this phenomenon. Finally, binding of TNP-proteins did not alter the ability of the B cells to present unrelated, unhaptenated proteins or to stimulate alloreactive T cells. These results suggest that surface Ig receptors serve to focus antigens onto specific B lymphocytes and that such cells are highly efficient at presenting linked antigenic determinants to T cells. The implications of these findings for the mechanisms of physiologic, histocompatibility-restricted T-B collaboration are discussed.

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