B-lymphocyte responses to trinitrophenyl-conjugated Ficoll: Requirement for T lymphocytes and Ia-bearing adherent cells

(antigen-presenting cells/thymus-independent antigens/helper T lymphocytes)

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ABSTRACT These studies were done to characterize the cellular requirements for B-lymphocyte responses to the haptenated polysaccharide trinitrophenyl-conjugated Ficoll. By using an *in vitro* microculture system, it was demonstrated that hapten-specific anti-trinitrophenyl-conjugated Ficoll plaque-forming cell responses by B lymphocytes require Ia-bearing adherent accessory cells and Thy 1⁺ Lyt 1⁺2⁻ nylon wool-nonadherent (T) lymphocytes. Such T cells could be primed *in vivo* with nonderivatized Ficoll to show carrier-specific helper cell function for derivatized Ficoll responses *in vitro*. The implications of these findings for our understanding of B-lymphocyte triggering by so-called T-independent antigens are discussed.

Our understanding of the mechanisms by which antigens trigger B lymphocytes to produce antibodies has been to a great extent shaped by the subdivision of these antigens into two classes: thymus dependent (TD) and thymus independent (TI). TD antigens activate B cells only in the presence of T lymphocytes and Ia-bearing accessory cells or factors derived from such cells. TI antigens activate B cells by mechanisms less well established. Although immune responses to TI antigens have been thought to occur in the absence of T lymphocytes, recent studies have called into question the T-cell independence of the plaque-forming cell (PFC) responses to some TI antigens (1). An accessory cell requirement for B-cell triggering by TI antigens has been shown, but the characterization of that cell is not complete and whether it indeed acts to present antigen has not been fully resolved (2, 3).

The present experiments were done to characterize more precisely the nature of the T-lymphocyte and accessory cell requirements for developing PFC responses to TI antigens. The derivatized polysaccharide trinitrophenyl (Tnp)-Ficoll was used as a prototypic TI antigen. We first show that Ia-bearing adherent cells are required for triggering B-cell responses to Tnp-Ficoll. We then demonstrate a T-cell requirement for *in vitro* PFC responses to Tnp-Ficoll and show that Ficoll-specific helper cells are present in splenic T-cell populations of appropriately primed mice.

MATERIALS AND METHODS

Mice. Female BALB/c mice were purchased from the Charles River Breeding Laboratories. They were maintained in our animal facilities and used when 8-20 weeks old.

Antigens. Poly(Glu⁶⁰Ala³⁰Tyr¹⁰) (all L; *M_r* 30,000-50,000; Vega Biochemicals) was dissolved in saline/1% Na₂CO₃, and the solution was adjusted to pH 7.0. Ficoll 400 (Pharmacia) was dissolved in phosphate-buffered saline. Tnp-(Glu⁶⁰Ala³⁰Tyr¹⁰)_n was prepared as described (4). This conjugate contained ≈1 mol

of Tnp per mol of (Glu⁶⁰Ala³⁰Tyr¹⁰)_n. Tnp-Ficoll was prepared by treating 5 mg of AECM⁹⁵-Ficoll (Biosearch, San Rafael, CA) in 0.5 ml of 0.2 M NaHCO₃, pH 9.0, with 0.1 ml of 2,4,6-trinitrobenzenesulfonic acid (7 mg/ml), stirring at 4°C for 18 hr, then dialyzing against phosphate-buffered saline, pH 8.0. This conjugate contained 86 Tnp groups per 400,000 daltons of AECM-Ficoll.

Immunizations. For priming splenic T cells, mice were immunized intraperitoneally 4 days before use with 25 μg of Ficoll, 25 μg of (Glu⁶⁰Ala³⁰Tyr¹⁰)_n in Maalox (5% of total volume)/pertussis vaccine (10% of total volume) (MP; Michigan Department of Public Health, Lansing, MI) (MP), or with MP alone.

Culture Conditions. Cultures were performed as described (4), using 5 × 10⁵ responding spleen cells in 200-μl volumes in flat-bottom wells of microtiter plates (Linbro) at 37°C in humidified 5% CO₂/95% air. Adherent cells, T cells, and antigens were added as noted. Six wells were plated for each culture and, after 4 days of culture, duplicate wells were pooled in Hanks' balanced salt solution for assay.

PFC Assay. The direct (IgM) anti-Tnp PFC response was assayed by a slide modification of the Jerne hemolytic plaque assay as described (4).

Antiserum. A xenogeneic monoclonal anti-Ia antibody, M5/114, with known reactivity for the *H-2^d* haplotype (A. Bhattacharya, personal communication) was the gift of Timothy Springer of this department. Adherent cells (2 × 10⁷) were incubated with 1 ml of hybridoma supernatant for 30 min at 4°C. Anti-Thy 1.2 treatments were performed with a twice-precipitated (NH₄)₂SO₄ fraction of culture supernatant from the hybridoma HO 13.4 (Salk Institute) (5). Three hundred micrograms of protein was incubated with 75 × 10⁶ whole spleen cells for 30 min at 4°C. Anti-Lyt 1.2 treatments were performed with a monoclonal antibody (New England Nuclear); 50 × 10⁶ nylon-nonadherent spleen cells were incubated in 0.5 ml of reagent diluted 1:100. Anti-Lyt 2.2 treatments were done with antibody containing ascitic fluid from the hybridoma clone ADH 4(15), the gift of Paul Gottlieb, University of Texas, Austin; 50 × 10⁶ nylon-nonadherent spleen cells were incubated in 0.5 ml of fluid diluted 1:20. After incubation with antibody, the cells were washed once. Complement (C) treatments for these antisera were then carried out for 45 min at 37°C with 1.0 ml of Low-Tox-M rabbit complement (Cedarlane Laboratories, Hornby, ON, Canada) diluted 1:5 in minimal essential medium. After C treatment, cells were washed three times before use in culture.

Abbreviations: C, complement; MP, Maalox/pertussis vaccine; PFC, plaque-forming cell; RAMB, rabbit anti-mouse brain; RAMG, rabbit anti-mouse immunoglobulin; SAC, splenic adherent cell, TD, thymus dependent; TI, thymus independent; Tnp, trinitrophenyl.

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Cell Fractionations. Sephadex G-10 passage of spleen cells to remove adherent cells was performed as described (4) using 10^7 cells per 10 ml of beads. Two-hour glass-adherent cells were prepared as described (4) and treated with 1500 rads of γ radiation (^{137}Cs source; 1 rad = 1.0×10^{-2} gray) before use in cultures. Nylon wool-passed T-cell-enriched populations were made according to the method of Julius *et al.* (6). Surface immunoglobulin-negative cells were prepared by passing spleen cells over antibody-coated polystyrene dishes as described (7).

RESULTS

Primary *in Vitro* PFC Responses to Tnp-Ficoll Occur over a Wide Range of Antigen Concentrations and Cell Densities. A microculture system was used to allow functional analysis *in vitro* of subsets of cells involved in the immune response to Tnp-Ficoll. As shown in Table 1, the magnitude of the primary PFC responses *in vitro* to Tnp-Ficoll varied little over a 100-fold range of concentration of stimulating antigen. The magnitudes of the responses to Tnp-Ficoll were also comparable when splenocytes were cultured at 5, 7.5, or 10×10^5 /well. Thus, neither the antigen concentration nor the cell density of the cultured spleen cells in the range investigated seem critical in determining the magnitude of the PFC response *in vitro* to Tnp-Ficoll. Thus, our experiments were done under culture conditions that we found to optimize PFC responses *in vitro* to Tnp-Ficoll.

***In Vitro* PFC Responses to Tnp-Ficoll Require Ia-Bearing Splenic Adherent Cells.** A requirement for Ia-bearing splenic adherent cells (SACs) for *in vitro* PFC responses to Tnp-Ficoll has been shown by Boswell *et al.* (8). This issue was reexamined under our experimental conditions. As shown in Table 2, a single passage of the responding cell population over a Sephadex G-10 column to remove adherent accessory cells eliminates 80% of the primary PFC response to Tnp-Ficoll. Adding untreated or C-alone-treated SACs to this Sephadex-passed responder population reconstitutes a PFC response to Tnp-Ficoll well above the response of normal spleen cells to the antigen. Yet treatment of the SAC with a monoclonal anti-Ia antibody and C markedly reduces the ability of these cells to reconstitute a PFC response to Tnp-Ficoll. Thus, SACs bearing Ia determinants are required for primary *in vitro* PFC responses to Tnp-Ficoll.

***In Vitro* PFC Responses to Tnp-Ficoll Are T-Cell Dependent.** Ia-bearing accessory cells play a critical role in the immune response to TD antigens. Our demonstration of an Ia-bearing SAC requirement for *in vitro* PFC responses to Tnp-Ficoll prompted us to look carefully at this response for a possible T-cell dependence. Table 3 summarizes experiments that establish a T-cell requirement for the primary *in vitro* PFC re-

Table 2. SACs bearing Ia determinants are required for primary *in vitro* PFC responses to Tnp-Ficoll

Responding cells	SACs		PFCs,* no. per 10^6 cultured cells
	Treatment	No. per culture	
Normal	—	—	184 ± 1.34
Normal, Sephadex G-10 passed	—	—	38 ± 1.77
	None	4×10^4	449 ± 1.20
		2×10^4	245 ± 1.41
	C alone	4×10^4	277 ± 1.40
		2×10^4	318 ± 1.31
	Anti-Ia and C	4×10^4	41 ± 2.26
		2×10^4	64 ± 1.84

Responding cells (5×10^5 per well) were cultured with Tnp-Ficoll (1 ng/ml) and cell populations as noted.

* Data are geometric mean $\pm$ SEM of parallel triplicate culture groups, each group a pool of two culture wells.

sponse to Tnp-Ficoll. Consistent with the recently published data of Mond *et al.* (1), a single treatment of the responding cell population with anti-Thy 1.2 and C essentially eliminates the PFC response to Tnp-Ficoll. The reduction in PFC responses to Tnp-Ficoll in responding cell populations after such treatments was 70–100%. We also observed this T-cell requirement when the responding cells were treated with rabbit anti-mouse brain (RAMB) and C (data not shown). We demonstrated the T-cell requirement for the *in vitro* PFC response to Tnp-Ficoll by reconstituting the response of anti-Thy 1.2 and C-treated splenocytes with nylon wool-passed spleen cells. As shown in Table 4, spleen T cells that had been depleted of surface Ig⁺ cells on rabbit anti-mouse immunoglobulin (RAMG)-coated dishes also reconstituted a response to a level well above that of the C-alone-treated unfractionated spleen cell population. That the reconstitution of the PFC response does not occur through optimization of macrophage density in the culture wells is shown by the lack of a significant difference in reconstituted PFC responses when comparing RAMG plate-purified T cells with similarly prepared cells that had been passed over a Sephadex G-10 column. That a significant PFC response did not come solely from the T-cell population is shown by the lack of a significant PFC response from either the RAMG plate-purified or nylon wool-passed spleen cells alone.

A Lyt 1⁺ T Cell Is Required for *in Vitro* PFC Responses to Tnp-Ficoll. We next attempted to differentiate the Thy 1-bearing cell required for responses to Tnp-Ficoll *in vitro* from the Thy 1-bearing cell that participates in responses to usual TD antigens. As shown in Table 5, the Lyt phenotype of the T cells required for *in vitro* PFC responses to Tnp-Ficoll and Tnp-(Glu⁶⁰Ala³⁰Tyr¹⁰)_n does not differ. Treatment of the reconstituting nylon-passed BALB/c splenocytes with anti-Lyt 1.2 and C eliminated the capacity of such cells to reconstitute PFC responses to Tnp-(Glu⁶⁰Ala³⁰Tyr¹⁰)_n and Tnp-Ficoll. A Lyt 1⁺ cell is therefore required for such responses. Anti-Lyt 2.2 and C-treated splenic T cells reconstituted both responses but not as efficiently as C-alone-treated splenic T lymphocytes. This suggests that (i) Lyt 1⁺2⁺ cells alone are sufficient for anti-Tnp-Ficoll responses *in vitro* and (ii) a Lyt 1⁺2⁺ cell may participate in such *in vitro* responses as well.

Ficoll Immunization Results in Carrier-Specific Priming of T Cells. Having demonstrated a T-cell requirement for the *in vitro* PFC response to Tnp-Ficoll, we sought to define the nature of the T-cell response to that antigen and, in particular, to

Table 1. Primary *in vitro* PFC responses to Tnp-Ficoll at various antigen concentrations and cell densities

Responding cells, no.	Tnp-Ficoll, ng/ml	PFCs,* no. per 10^6 cultured cells
Experiment 1		
5×10^5	10	132 ± 1.28
	1	132 ± 1.25
	0.1	145 ± 1.22
Experiment 2		
5×10^5	1	157 ± 1.22
7.5×10^5		125 ± 1.20
10×10^5		87 ± 1.15

* Data are geometric mean $\pm$ SEM of parallel triplicate culture groups, each group a pool of two culture wells.

Table 3. Primary IgM PFC responses *in vitro* to Tnp-Ficoll are T-cell dependent

Responding cells (no.)	PFCs,* no. per 10 ⁷ cultured cells			
	Exp. 1	Exp. 2	Exp. 3	Exp. 4
C alone treated (5 × 10 ⁵)	83 ± 1.24	263 ± 1.37	106 ± 1.16	73 ± 1.00
Anti-Thy 1.2 and C treated (5 × 10 ⁵)	1 ± 1.41	3 ± 1.95	1 ± 1.44	9 ± 1.72
Nylon wool passed (4 × 10 ⁵)	3 ± 2.15	1 ± 1.91	3 ± 1.36	2 ± 1.64
Anti-Thy 1.2 and C treated (4 × 10 ⁵) and nylon wool passed (1 × 10 ⁵)	180 ± 1.05	208 ± 1.09	137 ± 1.19	105 ± 1.05

Responding cells were cultured with Tnp-Ficoll (1 ng/ml).

* Data are geometric mean ± SEM of parallel triplicate culture groups, each group a pool of two culture wells.

determine whether T cells recognize the polysaccharide Ficoll as a carrier. We approached this question by examining the IgM PFC response of unprimed anti-Thy 1.2 and C-treated spleen cells to Tnp-Ficoll after reconstitution with Ficoll-primed T cells. As shown in Table 6, Ficoll-primed T cells reconstitute an anti-Tnp-Ficoll PFC response of twice the magnitude seen when adjuvant-alone-primed T cells are used to reconstitute such a response. The response seen with Ficoll-primed T cells is somewhat lower than that seen after reconstitution with Tnp-Ficoll-primed T cells.

That the enhancement of the IgM PFC response to Tnp-Ficoll with Ficoll-primed T cells is carrier specific is shown by the experiments in Table 7. Nylon wool-passed splenocytes from 4-day Ficoll-primed mice enhanced the PFC response to Tnp-Ficoll of anti-Thy 1.2 and C'-treated normal spleen cells when compared with the response reconstituted with adjuvant alone or with (Glu⁶⁰Ala³⁰Tyr¹⁰)_n-primed T cells. Yet there was no difference in the PFC response to Tnp-(Glu⁶⁰Ala³⁰Tyr¹⁰)_n reconstituted by either the adjuvant alone or Ficoll-primed T-cell populations when added to normal B cells. The PFC response

to Tnp-(Glu⁶⁰Ala³⁰Tyr¹⁰)_n reconstituted by the (Glu⁶⁰Ala³⁰Tyr¹⁰)_n-primed T cells was enhanced, though, when compared with responses reconstituted by other T-cell populations. Thus, Ficoll immunization causes a significant carrier-specific priming of T cells.

DISCUSSION

These studies were done to characterize the cellular requirements for B-lymphocyte responses to Tnp-Ficoll. We have shown that anti-Tnp-Ficoll PFC responses by B lymphocytes require Ia-bearing accessory cells and Thy 1⁺, Lyt 1⁺2⁻ T cells. It was further shown that these T cells could be primed by Ficoll to show carrier-specific helper function.

In vitro PFC responses to hapten derivatives of the synthetic polysaccharide polymer Ficoll were described as "T independent" when Mosier *et al.* (9) showed that splenocytes from congenitally athymic mice, anti-Thy 1.2 and C-treated normal splenocytes, and splenocytes from adult thymectomized mice all develop IgM PFC responses to Ficoll derivatives in culture. Boswell *et al.* (2), using an *in vitro* culture system similar to ours, have, in fact, recently reported that RAMB and C treatment of responding splenocytes does not abrogate and addition of purified T cells does not augment the *in vitro* IgM PFC response to Tnp-Ficoll. Yet, by treating responding splenocytes with a monoclonal anti-Thy 1.2 and C or with RAMB and C, we were able to markedly reduce the *in vitro* PFC response to this haptenated polysaccharide. Further, we were able to generate a PFC response to Tnp-Ficoll well above that seen in cultures of unfractionated spleen cells by adding back splenic T cells to the T-cell depleted cultures. These findings agree with the recent studies of Mond *et al.* (1, 10), which report similar T-cell requirements for *in vitro* PFC responses to Tnp-Ficoll, Tnp-polyacrylamide beads, and perhaps Tnp-*Brucella abortus*. That we have been able to define a T-cell requirement for *in vitro* Tnp-Ficoll PFC responses, where many previous workers were unable to do so, may well be due to the conditions under which these studies were performed: the cultures were carried out in wells of microtiter plates using a small number of responding cells and performing anti-Thy 1.2 and C treatment with a high-titer monoclonal antibody preparation.

Hapten-derivatized Ficoll has been classified as a type II TI antigen on the basis of its failure to elicit a response in mice that have the CBA/N X-linked defect and its inefficient stimulation of an immune response in neonatal mice (11). T-cell regulation of another type II TI antigen, type III pneumococcal polysaccharide, has previously been reported (12, 13). Immune responses to this antigen can be enhanced by prior treatment with antilymphocyte serum (12), and a T-cell-mediated state of low dose tolerance to this antigen can be achieved (13). Taken together with our demonstration that T cells can be primed to Ficoll, we may well raise the possibility that responses to at least TI type II antigens, when examined carefully, will be shown to

Table 4. Cells that reconstitute the PFC response to Tnp-Ficoll in T-cell-depleted cultures are not SACs

Responding cells		T cells		Direct PFCs,* no. per 10 ⁶ culture cells
		No. per culture	Treatment	
5 × 10 ⁵	None	—	—	198 ± 1.56
4 × 10 ⁵	Anti-Thy 1.2 and C	—	—	63 ± 1.23
		2.0 × 10 ⁵	RAMG plate purified	618 ± 1.07
		1.0 × 10 ⁵		444 ± 1.09
		0.5 × 10 ⁵		247 ± 1.19
		2.0 × 10 ⁵	Sephadex G-10 passed, then RAMG plate purified	445 ± 1.11
		1.0 × 10 ⁵		418 ± 1.15
		0.5 × 10 ⁵		252 ± 1.14
		2.0 × 10 ⁵	Nylon passed	389 ± 1.12
		1.0 × 10 ⁵		334 ± 1.33
		0.5 × 10 ⁵		178 ± 1.10
		5 × 10 ⁵	RAMG plate purified	1 ± 1.44
		5 × 10 ⁵	Nylon passed	9 ± 2.24

Responding cells were cultured with Tnp-Ficoll (1 ng/ml).

* Data are geometric mean ± SEM of parallel triplicate culture groups, each group a pool of two culture wells.

Table 5. T cells required for *in vitro* PFC responses to Tnp-Ficoll are Lyt 1⁺

Responding cells		T cells		PFCs,* no. per 10 ⁶
No.	Treatment	Treatment [†]	No. per culture	cultured cells
5 × 10 ⁵	None	Tnp-(Glu ⁶⁰ Ala ³⁰ Tyr ¹⁰) _n	—	156 ± 1.32
	Anti-Thy 1.2 and C	—	—	0
4 × 10 ⁵	Anti-Thy 1.2 and C	C alone	2 × 10 ⁵	123 ± 1.25
			1 × 10 ⁵	20 ± 2.30
		Anti-Lyt 1.2 and C	2 × 10 ⁵	4 ± 1.51
			1 × 10 ⁵	0
		Anti-Lyt 2.2 and C	2 × 10 ⁵	57 ± 1.19
			1 × 10 ⁵	37 ± 1.37
		Anti-Lyt 1.2 and C	1 × 10 ⁵ }	44 ± 1.74
		Anti-Lyt 2.2 and C	1 × 10 ⁵ }	
		C alone	5 × 10 ⁵	0
5 × 10 ⁵	Untreated	Tnp-Ficoll	—	118 ± 1.37
	Anti-Thy 1.2 and C	—	—	0
4 × 10 ⁵	Anti-Thy 1.2 and C	C alone	2 × 10 ⁵	92 ± 1.32
			1 × 10 ⁵	20 ± 1.11
		Anti-Lyt 1.2 and C	2 × 10 ⁵	3 ± 2.20
			1 × 10 ⁵	3 ± 1.82
		Anti-Thy 2.2 and C	2 × 10 ⁵	50 ± 1.37
			1 × 10 ⁵	70 ± 1.05
		Anti-Thy 1.2 and C	1 × 10 ⁵ }	59 ± 1.46
		Anti-Thy 2.2 and C	1 × 10 ⁵ }	
		C alone	5 × 10 ⁵	0

Responding cells were cultured with Tnp-Ficoll (1ng/ml) or Tnp-(Glu⁶⁰Ala³⁰Tyr¹⁰)_n (100 μg) as noted.

* Data are geometric mean ± SEM of parallel triplicate culture groups, each group a pool of two culture wells.

[†] In a BALB/c anti-C57BL/6J mixed lymphocyte reaction, C-alone-treated responders incorporated 157,000 cpm of [³H]thymidine, anti-Lyt 1.2-treated responders incorporated 1300 cpm, and anti-Lyt 2.2-treated responders incorporated 125,000 cpm. Cytolytic T lymphocytes generated in mixed lymphocyte reactions lysed C57BL/6J target blast cells at an effector/target ratio of 80:1 as follows: C-alone-treated, 45%; anti-Lyt 1.2-treated, 52%; anti-Lyt 2.2-treated, 10%.

involve essentially the same types of T-cell, accessory cell, and B-cell interactions as responses to classical TD antigens.

Our demonstration that T cells can be primed to the polysaccharide carrier Ficoll may throw into new perspective the experiments of Schott and Merchant (14). They demonstrated carrier-specific memory to Ficoll in congenitally athymic mice and ascribed that to a memory capacity in B cells. It is possible, in light of our studies, that the Ficoll-specific memory they described resides also in Thy 1-bearing cells, known to be present in *nude* mice in small numbers (15) or in a T-lymphocyte-like cell. We have, in fact, recently shown that the *in vitro* PFC response to Tnp-Ficoll of splenocytes from BALB/c *nude* mice, although unaffected by anti-Thy 1.2 and C or anti-Lyt 1.2 and

C-treatments, is dependent on a nylon-nonadherent cell population (unpublished observation).

The precise role played by the Ia-bearing accessory cell in the induction of an *in vitro* response to Tnp-Ficoll remains unresolved. Boswell *et al.* have reported that treatment of SACs with anti-Ia and C abrogates the capacity of that cell population to reconstitute the PFC response to Tnp-Ficoll of Sephadex G-10-passed splenocytes (8), a finding we have confirmed. This result, in the context of the observation of Boswell *et al.* that SACs pulsed with Tnp-Ficoll reconstitute a PFC response when added to an adherent cell-depleted population (2), would imply that Ia-bearing SACs are required for presentation of Tnp-Ficoll *in vitro*. Our observations lend further support to the position

Table 6. Ficoll-primed T cells help IgM PFC response *in vitro* to Tnp-Ficoll

Responding cells		T cells		PFCs,* no. per 10 ⁶ cultured cells	
No.	Treatment	No. per culture	Priming	Exp. 1	Exp. 2
5 × 10 ⁵	None	—	—	31 ± 1.20	68 ± 1.44
5 × 10 ⁵	Anti-Thy 1.2 and C	—	—	0	0
4 × 10 ⁵	Anti-Thy 1.2 and C	2 × 10 ⁵	MP alone	46 ± 1.63	32 ± 1.48
		1 × 10 ⁵		11 ± 2.39	5 ± 1.91
		2 × 10 ⁵	Ficoll (10 μg)/MP	85 ± 1.17	68 ± 1.36
		1 × 10 ⁵		56 ± 1.55	8 ± 1.39
		2 × 10 ⁵	TNP-Ficoll (10 μg)/MP	130 ± 1.31	146 ± 1.21
		1 × 10 ⁵		34 ± 1.30	43 ± 1.16
5 × 10 ⁵	Nylon passed, MP alone primed			0	—
5 × 10 ⁵	Nylon passed, Ficoll/MP primed			0	—
5 × 10 ⁵	Nylon passed, Tnp-Ficoll/MP primed			0	—

Responding cells (5 × 10⁵ per well) were cultured with Tnp-Ficoll (1 ng/ml) and cell populations as noted.

* Data are geometric mean ± SEM of parallel triplicate culture groups, each group a pool of two culture wells.

Table 7. Ficoll-primed T cells give carrier-specific help for *in vitro* IgM PFC responses to Tnp-Ficoll

Responding cells		T cells			PFCs,* no. per 10 ⁶ cultured cells		
No.	Treatment	No. per culture	Priming		Exp. 1	Exp. 2	Exp. 3
5 × 10 ⁵	None	—	—	Tnp-Ficoll	237 ± 1.14	46 ± 1.69	182 ± 1.22
5 × 10 ⁵	Anti-Thy 1.2 and C	—	—		3 ± 1.36	3 ± 1.17	45 ± 1.67
4 × 10 ⁵	Anti-Thy 1.2 and C	2 × 10 ⁵	MP alone		57 ± 1.13	34 ± 1.73	ND
		0.5 × 10 ⁵			5 ± 1.63	26 ± 1.19	ND
		2 × 10 ⁵	Ficoll (25 µg)/MP		98 ± 1.13	96 ± 1.18	269 ± 1.29
		0.5 × 10 ⁵			34 ± 1.34	83 ± 1.32	194 ± 1.22
		2 × 10 ⁵	(Glu ⁶⁰ Ala ³⁰ Tyr ¹⁰) _n (25 µg)/MP		63 ± 1.15	ND	182 ± 1.14
		0.5 × 10 ⁵			4 ± 2.39	ND	71 ± 1.75
5 × 10 ⁵	None	—	—	Tnp-(Glu ⁶⁰ Ala ³⁰ Tyr ¹⁰) _n	106 ± 1.55	46 ± 1.90	96 ± 1.18
5 × 10 ⁵	Anti-Thy 1.2 and C	—	—		1 ± 1.26	2 ± 1.86	0
4 × 10 ⁵	Anti-Thy 1.2 and C	2 × 10 ⁵	MP alone		28 ± 1.35	82 ± 1.05	ND
		0.5 × 10 ⁵			22 ± 1.97	73 ± 1.39	ND
		2 × 10 ⁵	Ficoll (25 µg)/MP		52 ± 1.41	88 ± 1.52	—
		0.5 × 10 ⁵			24 ± 1.91	24 ± 1.27	16 ± 1.23
		2 × 10 ⁵	(Glu ⁶⁰ Ala ³⁰ Tyr ¹⁰) _n (25 µg)/MP		156 ± 1.31	ND	—
		0.5 × 10 ⁵			153 ± 1.51	ND	200 ± 1.30

The data in each experiment shown in Tables 6 and 7 were normalized, the means were determined, and *P* values were calculated by Student's *t* test. The PFC response reconstituted by 2 × 10⁵ Ficoll/MP-primed T cells was greater than that reconstituted by an equal number of MP-alone-primed T cells (*P* = 0.003). Responding cells were cultured with Tnp-Ficoll (1 ng/ml) or Tnp-(Glu⁶⁰Ala³⁰Tyr¹⁰)_n (100 µg/ml) as noted. ND, not determined.

* Data are geometric mean ± SEM of parallel triplicate culture groups, each group a pool of two culture wells.

that Ia-bearing accessory cells are critical for *in vitro* PFC responses to Tnp-Ficoll but do not directly demonstrate antigen presentation by such cells. The similarity between the cellular requirements for *in vitro* PFC responses to Tnp-Ficoll and those for responses to usual TD antigens suggests that Ficoll presentation to T lymphocytes by accessory cells may well occur in an *I*-region-restricted interaction. We cannot, of course, preclude the possibility that accessory cell-B lymphocyte interactions occur in an *I*-region-restricted fashion as suggested by Boswell *et al.* (8).

The data presented here suggest that a strict separation of antigens into TI and TD may not be possible. Perhaps more consistent with the accumulating data in this area is the concept that a continuum of antigens exists, extending from those that have a large T-cell requirement for B-cell triggering to those that have a very small requirement. Further work with the so-called TI antigens should clarify these issues.

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