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Immunology

In vitro immune responses to a T cell-dependent antigen by cultures of disassociated murine Peyer's patch

(macrophages/neutral protease/anti-sheep erythrocyte responses/IgA responses/adjuvancy)

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ABSTRACT The first line of defense against pathogens that enter the host by the oral route appears to involve the gut-associated lymphoreticular tissues—e.g., Peyer's patches (PP). Although animals can readily be immunized by orally administered antigen that mobilizes the secretory immune system, there is a total lack of local antibody synthesis in the PP and the cellular basis for this deficiency remains a mystery. A lymphoreticular cell population, obtained when murine PP were treated with a neutral protease (Dispase), consisted of accessory cells [macrophages (MΦ)] and T and B lymphocytes. *In vitro* cultures of these PP cell preparations with the thymic-dependent antigen sheep erythrocytes (SRBC) resulted in good anti-SRBC plaque-forming cell (PFC) responses. The time courses of these responses were identical to those seen with spleen cell cultures. Submitogenic concentrations of concanavalin A (Con A) and optimal doses of *N*-acetylmuramyl-L-alanyl-D-isoglutamine (MDP) and lipopolysaccharide (LPS) enhanced *in vitro* responses of PP cell cultures to SRBC. PP possess fully functional antigen-presenting MΦ because incubation of optimal proportions of splenic T and B cells with purified populations of PP MΦ supported good *in vitro* immune responses. Murine PP possess all of the necessary elements for an IgA immune response because PP cell cultures derived from mice orally primed with SRBC and immunized with SRBC *in vitro* gave high IgA anti-SRBC PFC responses. All of the adjuvants tested (LPS, MDP, and Con A) enhanced IgA responses in PP cell cultures from orally primed mice; however, Con A induced the greatest enhancement. These results demonstrate that murine PP possess MΦ capable of accessory cell functions for *in vitro* immune responses and that oral priming with antigen induces the precursor T- and B-cell populations necessary for IgA responses, that are potentiated by adjuvants, in PP cell cultures. Thus, murine PP possess the lymphoreticular cells required for antibody responses; however, the tissue architecture likely prevents local responses *in vivo*. The finding that enzymatically dissociated PP contain all of the necessary cellular components for antibody synthesis, whereas the *in vivo* tissue architecture prevents the complex interactions necessary for this response, suggests that the initial inductive events take place *in situ*, and additional cell interactions are required for final differentiation of IgA-synthesizing plasma cells to occur at distant mucosal sites.

Peyer's patches (PP) contain antigen-sensitive T and B cells (1) as well as macrophages (MΦ) (2) and are covered by an actively pinocytotic epithelium that readily delivers adsorbed gut lumenal antigens to underlying lymphoreticular cells. Thus, one would predict that oral administration of antigen would induce specific immune responses and antibody production in this gut-associated lymphoreticular tissue (GALT). Although oral immunization sensitizes both T cells (3, 4) and precursor IgA B cells (5) in GALT, local antibody production does not occur (6, 7). Instead, sensitized lymphocytes leave PP via efferent lymphatics, enter the blood circulation, and selectively populate mucosal sites (8, 9). The lack of local antibody synthesis in PP has been attributed to a deficiency (or architectural separation) of at least one of the three cell types required for immune responses to thymic-dependent (TD) antigens—namely, T and B lymphocytes and macrophages (MΦ) (see ref. 10 for review).

Kagnoff and associates (1, 11, 12) have reported that dissociated PP cells lack an accessory (MΦ) cell population required for *in vitro* responses to TD antigens. PP from athymic, *nude* mice lacked both MΦ and T cells, and this B cell population was unresponsive to the B-cell mitogen lipopolysaccharide (LPS); responses to the mitogen were restored by exogenously supplied T cells and MΦ or 2-mercaptoethanol (11). Others have been unable to confirm that T cells are required for LPS mitogenic responses (13). Kagnoff (12) has further suggested that MΦ are not required for the induction of T helper (T_h) cells because PP cells from mice fed erythrocytes exhibited good T_h cell activity for *in vitro* immune responses. These studies (1, 11, 12) represent the major evidence for the lack of MΦ in PP which are necessary for *in vitro* immune responses to TD antigens.

We report here that enzymatically dissociated murine PP cell cultures contain functional T and B lymphocytes and accessory cells (MΦ) for *in vitro* immune responses to the TD antigen sheep erythrocytes (SRBC). Furthermore, PP cell cultures from mice orally primed with SRBC preferentially exhibit plaque-forming cell (PFC) responses of the IgA isotype. Adjuvants that affect one or more lymphoreticular cell types augment these *in vitro* immune responses.

MATERIALS AND METHODS

Mice and Oral Priming. BALB/c mice were bred and maintained in The Core Facility for Immunocompromised Mice, The Comprehensive Cancer Center at the University of Alabama, Birmingham. All mice used in these studies were 8–12 wk of age. For oral priming, mice were given SRBC via a gastric tube as described (4).

Isolation of Murine PP Cells. Murine PP were aseptically removed (4) and transferred to a Petri dish that contained a so-

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Abbreviations: PP, Peyer's patches; PFC, plaque-forming cell(s); MΦ, macrophages (general term used to describe accessory cells); LPS, lipopolysaccharide; MDP, *N*-acetylmuramyl-L-alanyl-D-isoglutamine (muramyl dipeptide); Con A, concanavalin A; SRBC, sheep erythrocytes; GALT, gut-associated lymphoreticular tissue; TD, thymic-dependent; T_h, T helper; FITC, fluorescein isothiocyanate; RITC, rhodamine isothiocyanate; LPS(Ph), *Escherichia coli* K235 LPS prepared by phenol/water extraction; LPS(Bu), *Escherichia coli* K235 LPS prepared by butanol/water extraction; HRBC, horse erythrocytes.

lution of Dispase (1.5 mg/ml; grade II, Boehringer Mannheim) in Joklik-modified (GIBCO) minimal essential medium as described (14). Dissociated cells were washed extensively and were resuspended to the appropriate concentration in complete medium (15, 16).

Characterization of PP Cells. Fluorescein isothiocyanate (FITC)-conjugated monoclonal anti-mouse Thy 1.2 (Becton-Dickinson, Sunnyvale, CA) and rhodamine isothiocyanate (RITC)-conjugated goat anti-mouse k + λ (a kind gift of John F. Kearney, Department of Microbiology, University of Alabama in Birmingham) were used to count the T and B cells in PP preparations. Individual PP cell suspensions (2×10^6 cells) were incubated (45 min, 4°C) with FITC anti-Thy 1.2 and RITC anti-mouse k + λ reagents and washed three times with phosphate-buffered saline that contained 1% bovine serum albumin. The cells stained with FITC- or RITC-labeled reagent were counted with an immunofluorescence microscope (Orthoplan, Leitz, Wetzlar, Federal Republic of Germany). Between 10 and 15 fields were counted per slide depending upon the number of cells present (i.e., at least 200 cells were counted).

Esterase-positive cells (17) with morphological characteristics compatible with MΦ were enumerated in PP preparations by counting the positive cells per 1000 total cells visualized with a light microscope (Leitz). Esterase-stained cells with morphological characteristics of epithelial cells or lymphocytes were excluded.

PP and Splenic-Adherent Accessory Cells. MΦ-enriched preparations from dissociated PP or spleen were obtained by an adherence technique (15). Spleen cell suspensions were prepared by teasing spleen cells through wire screen (60-mesh). Single-cell suspensions of spleen or PP were pelleted by centrifugation (450 × *g*, 10 min), irradiated (1500 rads; 1 rad = 0.01 J/kg = 0.01 Gy) in a Packard Vangard machine (280 kV, 20 mA under maximal backscatter conditions), and then washed twice in complete medium. After the final wash, cells were resuspended in complete medium and added (5×10^6/0.5 ml) to each well of Linbro macroculture plates (Linbro, Hamden, CT). Cells were allowed to adhere during incubation at 37°C for 2 hr and then nonadherent cells were removed by washing and aspiration. After the last wash, appropriate dilutions of lymphocytes were added for *in vitro* cultures (described below).

To show that the adherent cell preparations from murine PP have characteristics associated with MΦ, the following criteria were used. Approximately 90% (87–92%) of these cells were esterase-positive and actively phagocytized latex particles and antibody-coated SRBC. For convenience, we have termed these cells MΦ; however, in this study we have not distinguished other adherent cell types—e.g., dendritic cells—that are present in murine PP (18).

Splenic T and B Lymphocytes. T and B lymphocytes were purified by passage of spleen cell suspensions through two consecutive Sephadex G-10 columns (15, 19). The eluted cells consisted of >99% lymphocytes that were 93–96% viable and did not support *in vitro* responses to SRBC.

***In Vitro* Immune Responses.** Single-cell suspensions of PP or spleen were cultured (5×10^6/0.5 ml) with antigen by using the method of Mishell and Dutton (16) with modifications (15). In separate experiments, cultures containing either PP or splenic MΦ were incubated with splenic T and B cells and SRBC ($2–3 \times 10^6$). In other experiments, appropriate concentrations of various immunopotentiating stimulants were added to PP and spleen cell cultures immunized with SRBC. All cultures were assessed for IgM anti-SRBC PFC responses. In some experiments, PP cell cultures from mice orally primed with SRBC were immunized with SRBC and assessed for the isotype of anti-SRBC responses.

Stimulants. *N*-acetylmuramyl-L-alanyl-D-isoglutamine (MDP) (Sigma) and concanavalin A (Con A) (Miles) were dissolved (1 mg/ml) in incomplete medium (15, 16), divided into portions, and stored at −20°C until used. *Escherichia coli* K235 LPS was prepared by a phenol/water extraction method [LPS(Ph)] (20) or by a butanol/water extraction procedure [LPS(Bu)](21).

PFC Assay. Nonadherent cells were removed from culture wells, washed, and resuspended in Hanks' balanced salt solution (GIBCO) to an appropriate volume for assay. Cultures were assessed in triplicate for anti-SRBC PFC by using the slide modification (16) of the Jerne hemolytic plaque assay. For measurement of IgG and IgA anti-SRBC PFC responses, slides were first incubated for 1.5 hr at 37°C in an atmosphere of 5% CO_2 in air; this was followed by an additional hour with optimal amounts of monospecific anti-mouse γ or α serum as described (4). Plaques were visualized after incubation with guinea pig complement. Total numbers of IgM, IgG, and IgA anti-SRBC PFC were determined in each culture.

Statistical Analysis. Values for the PFC assay are expressed as the mean PFC response per culture, ±SEM. The significance of difference between means was determined by Student's *t* test.

RESULTS

Characterization of Enzymatically Dissociated PP Cells. Enzymatic extraction of PP yielded 3- to 4-fold higher cell numbers than conventional mechanical methods (14, 22) and gave similar proportions of T and B lymphocytes, but >10-fold higher numbers of esterase-positive MΦ (Table 1).

Anti-SRBC PFC Responses in PP Cell Cultures. In our initial experiments, PP and spleen cell cultures were immunized with SRBC and assayed for anti-SRBC PFC responses on days 2–7 of culture (Fig. 1). The level of anti-SRBC PFC obtained in PP and spleen cell cultures increased significantly ($P < 0.01$) between days 3 and 4 of culture; maximal responses were obtained on day 5. Although spleen cell cultures yielded slightly higher anti-SRBC PFC responses, they were not significantly different ($P \leq 0.01$, except for day 6) from those obtained in PP cell cultures (Fig. 1). No IgG or IgA anti-SRBC PFC responses were observed in primary spleen or PP cell cultures (data not shown).

Our next experiments were directed toward determining whether known immunopotentiating agents—e.g., LPS (15, 23), MDP (24–27), or Con A (22)—would also enhance immune responses in PP cell cultures. In these studies, stimulants were added at the time of *in vitro* immunization. A submitogenic dose of Con A augmented immune responses in both PP and spleen cell cultures (Table 2). A similar potentiation was obtained with LPS(Ph) and LPS(Bu) and with MDP. These results demonstrate that adjuvants that act on T cells (Con A), MΦ (MDP or LPS), or T and B cells and MΦ (LPS and possibly MDP) are

Table 1. Distribution of T and B lymphocytes and MΦ in enzymatically dissociated PP cell preparations

Mouse strain	Immunofluorescence		Esterase-positive
	Anti-Thy 1.2	Anti-κ + λ	(MΦ)
BALB/c	35.5 ± 1.1	41.4 ± 1.4	7.7 ± 0.3
C3H/HeJ	37.4 ± 1.6	40.6 ± 2.1	6.5 ± 0.3
C3H/HeN	36.2 ± 0.8	41.2 ± 0.9	6.8 ± 0.5

PP cells were pooled from 7–10 mice per strain; triplicate slides were prepared and positive cells were enumerated in 10–15 fields. Values are the mean percentage ± SEM of three separate experiments.

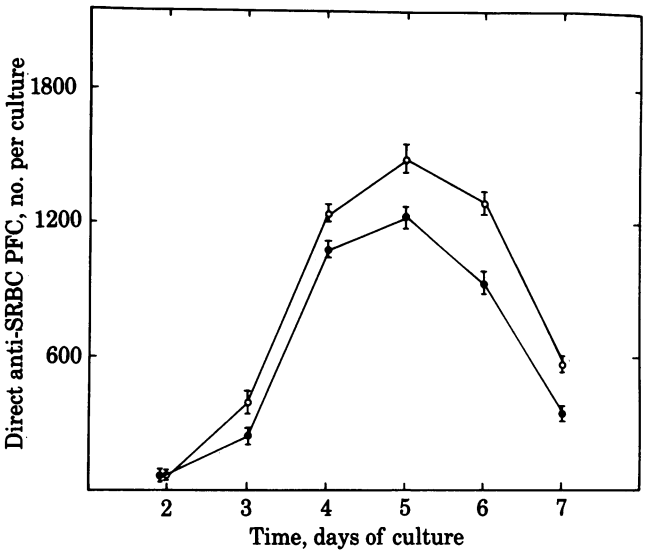

FIG. 1. *In vitro* immune response to SRBC of BALB/c spleen (○) and PP (●) cell cultures. Spleen cells (obtained by passage of cells through wire mesh) and PP cells (obtained by treatment of patches with Dispase) were cultured (5×10^6/0.5 ml of complete minimal essential medium) with SRBC ($2–3 \times 10^6$). Triplicate cultures were harvested daily and assessed for the number of direct (IgM) anti-SRBC PFC. Controls were anti-horse erythrocytes (HRBC) (range, 4–22) PFC and nonimmunized cultures (anti-SRBC range, 16–42 PFC per culture). Values are the mean ± SEM of four separate experiments.

effective in augmenting immune responses to SRBC in both PP and spleen cell cultures.

PP Macrophages Support *in Vitro* Immune Responses. These experiments were designed to prove that MΦ from PP could perform accessory cell functions necessary for *in vitro* immune responses. Splenic and PP MΦ were obtained and incubated with SRBC and various concentrations of purified splenic T and B cells (Table 3). Good anti-SRBC PFC responses were obtained in cultures that contained PP MΦ and splenic T and B lymphocytes. Optimal immune responses (not significantly different, $P \leq 0.01$) were obtained in cultures that contained PP or spleen MΦ derived from 5×10^6 total irradiated cells and 5×10^6 purified splenic T and B cells. These results demonstrate that PP MΦ possess accessory cells that support specific *in vitro* immune responses.

***In Vitro* IgA Immune Responses in PP Cell Cultures.** PP cell cultures from orally primed mice were incubated with SRBC

Table 2. Augmentation of *in vitro* immune response to SRBC by various adjuvants in PP and spleen cell cultures

Adjuvant added to culture, μg	IgM anti-SRBC, no. PFC per culture*			
	PP		Spleen	
	With SRBC	No SRBC	With SRBC	No SRBC
None	1198 ± 96	24 ± 4	1524 ± 121	18 ± 7
Con A, 1.25	1880 ± 116	44 ± 12	2892 ± 154	36 ± 9
LPS(Ph), 1	1921 ± 153	33 ± 12	2664 ± 161	42 ± 18
LPS(Bu), 1	2068 ± 97	41 ± 9	2994 ± 104	38 ± 11
MDP, 25	1846 ± 94	31 ± 6	2779 ± 52	29 ± 6

BALB/c spleen or PP cell cultures (5×10^6 cells per well) were incubated with (or without) SRBC ($2–3 \times 10^6$) and adjuvant (at the indicated concentrations).

* Anti-SRBC PFC was determined on day 5 of culture. Controls included anti-HRBC PFC responses; these ranged from 8 to 26 PFC in immunized or adjuvant-treated cultures. Values represent the mean ± SEM of triplicate determinations per experiment from three separate experiments.

Table 3. Capacity of PP MΦ to perform accessory functions for *in vitro* immune responses to SRBC

MΦ concentration*	IgM anti-SRBC PFC, no. per culture†				
	None	1.0	2.5	5.0	10.0
	MΦ from PP				
None	—	12 ± 4	23 ± 3	7 ± 3	13 ± 4
2.5	12 ± 3	435 ± 58	545 ± 7	435 ± 48	770 ± 43
5.0	15 ± 9	260 ± 18	820 ± 82	1274 ± 137	1035 ± 78
	MΦ from spleen				
None	—	7 ± 2	29 ± 3	14 ± 3	18 ± 6
2.5	18 ± 4	335 ± 39	385 ± 26	415 ± 27	215 ± 10
5.0	30 ± 6	445 ± 61	1340 ± 73	1515 ± 79	668 ± 67

Purified BALB/c mouse splenic T + B cells were incubated at the indicated concentrations with or without MΦ (from either spleen or PP) and with SRBC ($2–3 \times 10^6$).

* MΦ derived from PP or spleen given 1500 rads and plated at an equivalent (0, 2.5, or 5.0×10^6 cells per 0.5 ml) concentration were employed.

† Anti-SRBC PFC responses were determined in the absence of T and B cells and at four different concentrations of splenic T and B cells (1.0, 2.5, 5.0, and 10.0×10^6 per culture) on day 5 of culture; mean ± SEM of cultures in triplicate determinations per experiment from three separate experiments.

and the levels of IgM, IgG, and IgA anti-SRBC PFC were determined (Fig. 2). Maximal immune responses of all isotypes were obtained on days 4 and 5 of culture. The level of IgA anti-SRBC PFC was approximately 2-fold higher than that observed with IgM or IgG responses. These results demonstrate that oral priming with SRBC sensitizes PP for immune responses and that the IgA isotype response is favored. The results further suggest that PP possess the cell populations required for IgA immune responses.

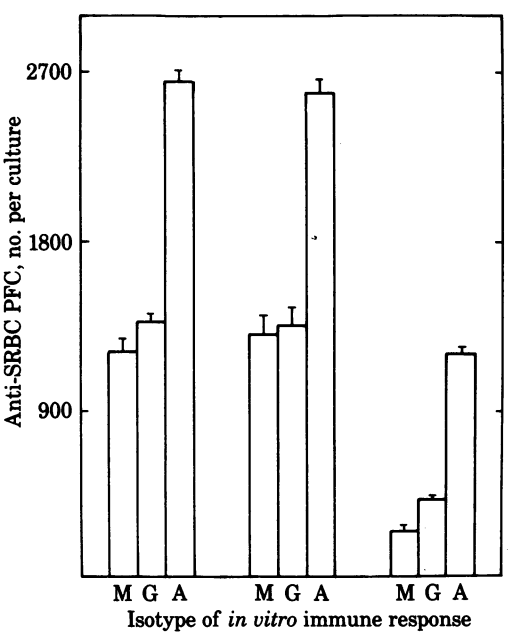

FIG. 2. *In vitro* immune response to SRBC of PP cell cultures from BALB/c mice orally primed with SRBC. PP cells were cultured (5×10^6/0.5 ml of complete minimal essential medium) with SRBC ($2–3 \times 10^6$). Triplicate cultures were harvested on days 4 (*Left*), 5 (*Middle*), and 6 (*Right*) of incubation and assessed for the number of direct [IgM (M)] and indirect [IgG (G) and IgA (A)] anti-SRBC PFC. Controls, including nonimmunized cultures, were: IgM, 22–48; IgG, 14–24; and IgA 20–44 anti-SRBC PFC per culture. Values are the mean ± SEM of five separate experiments.

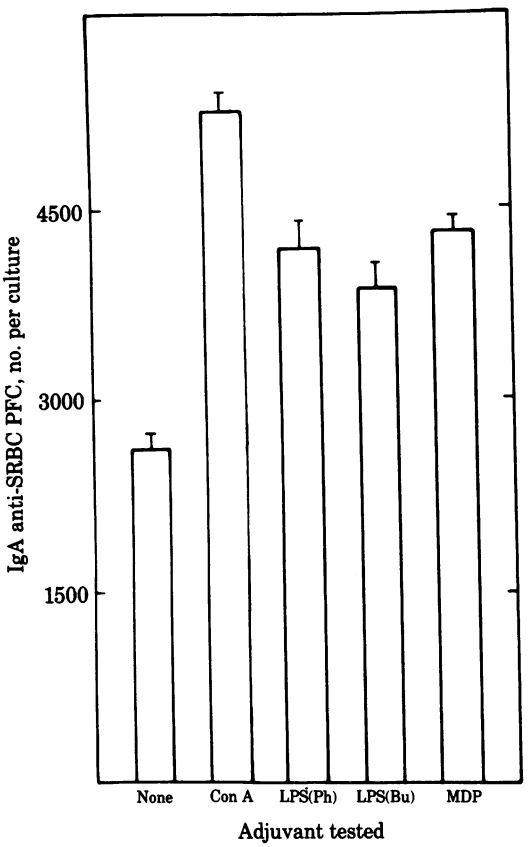

FIG. 3. Augmentation of *in vitro* IgA immune responses to SRBC of PP cell cultures. PP cells from SRBC orally primed BALB/c mice were cultured (5×10^6/0.5 ml of complete minimal essential medium) with SRBC ($2–3 \times 10^6$) and either Con A (1.25 μg per culture), LPS(Ph) (1.0 μg per culture), LPS(Bu) (1.0 μg per culture), or MDP (25 μg per culture). Triplicate cultures were harvested on day 5 of incubation and assessed for the number of IgA anti-SRBC PFC. The numbers of IgM anti-SRBC PFC were: 988 ± 64 (control, no adjuvant); 1340 ± 82 (Con A); 1455 ± 90 [LPS(Ph)]; 1515 ± 65 [LPS(Bu)]; and 1394 ± 102 (MDP). The numbers of IgG anti-SRBC PFC were: 1088 ± 110 (control, no adjuvant); 1940 ± 72 (Con A); 1775 ± 94 [LPS(Ph)]; 2010 ± 115 [LPS(Bu)]; and 1868 ± 96 (MDP). Values are the mean ± SEM of five separate experiments.

Because these results demonstrate *in vitro* IgA responses in murine PP cell cultures, it was of interest to determine whether this response could be augmented with adjuvants. Significant enhancement of IgA anti-SRBC PFC responses in PP cell cultures (from SRBC orally primed mice) was obtained with all adjuvants tested (Fig. 3). Moreover, Con A, at submitogenic doses, resulted in the highest IgA responses, suggesting that T_h cells or their mediators are of particular importance in the IgA immune response *in vitro*.

DISCUSSION

The present study has demonstrated that PP cell cultures that contained T_h cells, antigen-sensitized B cells, and MΦ are sufficient for IgA immune responses to the TD antigen SRBC. However, our results cannot explain why local immune responses do not occur in the PP to antigens encountered via the oral route. It is well known that distinct T- and B-cell zones are found in GALT (28, 29), and it is possible that MΦ are physically separated from areas where T and B cell interactions occur. It is tempting to speculate that antigen processing by MΦ in either T-cell or B-cell zones with subsequent presentation to T or B lymphocytes would result in initial induction of immune responses. The terminal inductive stimuli required for immune responses may occur in distant mucosal tissues after departure of these cells from PP. Additional work remains to be done to determine why T and precursor IgA B cells selectively migrate to distant mucosa and the microenvironmental conditions that determine final differentiation of B cells into fully mature plasma cells synthesizing IgA.

The method used for isolation of cells from the PP is of critical importance for recovery of the total lymphoreticular cell population. When the enzyme Dispase was employed, a lymphoreticular cell population was obtained that contained significant numbers of MΦ and T and B lymphocytes (Table 1). In this regard, Richman and coworkers (30) recently reported that collagenase treatment of PP yielded MΦ capable of presenting antigen to primed T cells; similar numbers of MΦ were also obtained without enzyme treatment. However, the ability of these latter cells to present antigen was diminished. Our own experience has been that conventional dissociation techniques yield populations rich in T and B cells but deficient in MΦ (22). The reason for this apparent discrepancy is unknown; however, in both cases, enzyme dissociation resulted in a functional MΦ population.

It is well known that *in vitro* immune responses to TD antigens are dependent upon a precise interaction of MΦ and T and B lymphocytes. Cell contact, MΦ, T cells, and their mediators are critical for antigen-specific induction of antibody-producing cells. A defect in one or more of these elements would prevent antigen-specific immune responses. However, our studies show that good *in vitro* responses to SRBC occur in PP cell cultures. Although responses were lower on a per cell basis than with parallel spleen cell cultures, significant anti-SRBC PFC responses were induced in cultures derived from enzyme-dissociated murine PP.

Our studies indicate a central role of the MΦ for immune responses in PP cultures and we have shown that sufficient accessory cells are present in the PP for support of *in vitro* immune responses. Kagnoff (12) originally suggested that MΦ may not be required for induction of T_h cells in PP because he found significant T_h cell activity without demonstrable MΦ in dissociated PP cell cultures from orally primed mice. We favor the view that MΦ are important for induction of T_h cells in PP; however, formal proof of this will require further experimentation.

A useful means of probing whether normal cell interactions occurred in PP cell cultures immunized with SRBC was provided by use of molecular immunostimulants that have been demonstrated in other *in vitro* situations to potentiate immune responses. Elson *et al.* (31) showed that PP T cells stimulated with Con A selectively promote IgA and suppress IgM and IgG isotype expression in LPS-stimulated PP cell cultures. In the present study, Con A, at submitogenic levels, enhanced *in vitro* immune responses of PP cell cultures immunized with SRBC. Moreover, this lectin induced 2-fold greater IgA anti-SRBC PFC responses in PP cell cultures from mice previously immunized gastrically. The data further suggest that Con A-stimulated T_h cells are important for the induction of IgA responses. Further work will be required to determine whether specific T_h cells and their mediators are necessary for IgA responses *in vitro*. It is possible that PP are an enriched source of T cells that collaborate with B cells for IgA responses.

Recent studies have shown that antigen-specific T_h cells for IgA responses can be induced in murine PP within 1 day after oral administration of protein antigen (32). The antigen form itself must also be considered in studies of *in vitro* IgA responses because significant *in vitro* IgA responses to the dextran B1355 have been reported (33). The type of response was both T cell- and age-dependent and prior treatment with anti-Thy 1.2 plus complement abrogated it. Because older mice were necessary

for IgA anti-B1355 responses, indigenous polyglucans in the gut may have environmentally primed these animals for subsequent immune responses (33).

Our studies indicated that MDP enhanced *in vitro* immune responses to SRBC in PP cell cultures. Watson and Whitlock (26) suggested that MDP may act as a T-cell replacing factor-like signal for B cells. Thus, the enhancement of IgA anti-SRBC PFC responses in PP cell cultures from SRBC orally primed mice could have resulted from a direct signal to precursor IgA B cells. This could be an oversimplification because it has been suggested that MDP acts directly on the MΦ and enhanced responses may result from MΦ mediator production [e.g., interleukin 1 (25)]. It should be noted that interleukin 1 does promote good immune responses in PP lymphocyte cultures (4).

Recent studies (see ref. 34 for review) have indicated that more than one lymphoreticular cell are important for LPS-adjuvant responses. Jacobs (35) suggested that LPS induces the B cell to become more sensitive to T cell-replacing factor, demonstrating that TRF and LPS act in a synergistic fashion on B cells responding to TD antigens. Hoffman *et al.* (23) suggested that MΦ are of central importance in LPS-induced enhancement of antibody production, whereas we have shown (15) that T cells and MΦ are both required for LPS adjuvancy. From the present study, it is clear that LPS enhances *in vitro* immune responses to SRBC in PP cell cultures. The LPS molecule also enhances IgA responses in PP cell cultures derived from orally primed mice.

Our studies provide evidence that the murine PP harbor a full repertoire of lymphoreticular cells—namely, T and B lymphocytes and accessory cells which are fully functional *in vitro*. This population exhibits complete potential for expression of *in vitro* immune responses to TD antigens. Furthermore, all of the elements necessary for IgA responses are present within this tissue. Well-characterized immunostimulants enhance these *in vitro* immune responses. It is now possible to determine the precise requirements for thymic-dependent antigen sensitization of precursor IgA B cells in GALT. The manner in which MΦ process and present antigen to lymphoid cells and the interaction of T_h cells and other regulatory circuits that direct the precursor B cell to mobilize to distant mucosal sites and subsequently differentiate into fully mature IgA synthesizing plasma cells are important areas to be investigated.

Note Added in Proof. Since the submission of this manuscript, we have initiated more extensive characterization of the accessory cell population in enzymatically dissociated PP cell preparations utilizing an oxidative mitogenesis system (periodate treatment). We have observed that PP accessory cell activity in this system resides predominantly in the nonadherent fraction. The precise relationship of this accessory cell population to that required for antibody formation remains to be elucidated.

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