FUNCTIONAL CHARACTERISTICS OF PEYER'S PATCH CELLS

III. Carrier Priming of T Cells by Antigen Feeding*

By MARTIN F. KAGNOFF‡

(From the Department of Medicine, University of California, San Diego, La Jolla, California 92037)

Murine Peyer's patches contain antigen-sensitive B and T cells but are naturally deficient in an accessory adherent cell type(s) required for the induction of both humoral antibody synthesis and cell-mediated cytotoxic reactions in vitro (1). After the addition of either syngeneic peritoneal adherent cells (PAC)[1] or 2-mercaptoethanol (ME) to culture, Peyer's patch B cells could be specifically induced to antibody-forming cells by erythrocyte antigens and Peyer's patch T cells could cooperate in B-cell induction as well as mediate cytotoxic allograft reactions against cell surface alloantigens (1). These studies suggested that Peyer's patches in mice contain antigen-sensitive B and T cells sequestered in such a way as to lack an accessory adherent cell type(s) or factor required for the induction of certain immune responses. In support of this concept several laboratories including our own could not demonstrate significant in vivo induction of Peyer's patch B cells to humoral antibody synthesis when erythrocyte (reference 2 and M. Kagnoff, unpublished observations) or protein (3) antigens were administered by either the oral or parenteral route.

These findings prompted us to ask whether oral administration of antigen leads to a priming of Peyer's patch antigen-sensitive cells in the absence of B-cell induction to humoral antibody synthesis. In these investigations we explored the possibility that oral administration of antigen might result in priming of T cells in Peyer's patches. Using a trinitrophenyl (TNP)-coupled erythrocyte system (4), we assessed the ability of Peyer's patch cells from mice fed erythrocytes to specifically enhance the in vitro anti-TNP response of normal Peyer's patch cells stimulated with TNP coupled to the homologous erythrocyte carrier. Our results indicate that Peyer's patch T cells can be specifically carrier primed for T-cell helper function by antigen feeding. This finding suggests a significant functional role for Peyer's patch T cells in vivo and may indicate that T-cell priming for helper function requires little, if any, cooperation from the macrophage-like accessory adherent cell.

Materials and Methods

Mice. 8- to 12-wk old hybrid C57BL/6 × DBA/2 mice (BDF_1) were obtained from Jackson Laboratories, Bar Harbor, Maine.

* Supported by National Institute of Arthritis, Metabolism and Digestive Diseases research grants AM-17276, AM-70283, and a grant from the National Foundation for Ileitis and Colitis.

‡ Recipient of Research Career Development Award 1 K04AM-00021 from the National Institute of Arthritis, Metabolism and Digestive Diseases.

[1] *Abbreviations used in this paper:* ADCC, antibody-dependent cell-mediated cytotoxic; BDF_1, C57BL/6 × DBA/2; BSA, bovine serum albumin; BSS, Hanks' balanced salt solution; CRBC, chicken erythrocytes; FBS, fetal bovine serum; HRBC, horse erythrocytes; ME, 2-mercaptoethanol; PAC, peritoneal adherent cells; PFC, plaque-forming cells; RAMB, rabbit antimouse brain; TNP, trinitrophenyl.

Antigens. Sheep (SRBC), chicken (CRBC), and horse erythrocytes (HRBC) were obtained from the Colorado Serum Co., Denver, Colo. 2,4,6-trinitrophenyl-coupled erythrocytes (TNP-SRBC or TNP-CRBC) were prepared as described by Rittenberg and Pratt (5). Heavily substituted erythrocytes (4) were used as the immunogen in culture.

Immunization in Vivo. For oral immunization, normal BDF_1 mice were permitted to drink ad libitum from a 2% vol/vol SRBC or CRBC suspension in Hanks' balanced salt solution (BSS) for 8 days before sacrifice. Additional normal BDF_1 mice drank no erythrocytes and served as a control group. For low carrier-dose priming (6), mice were injected intravenously into the lateral tail vein, or intraperitoneally with 0.2 ml of a 0.01% SRBC or CRBC suspension 6 days before sacrifice. Groups of three to five mice were fed or injected with erythrocytes in each experiment and the spleens and/or Peyer's patches of each group were pooled for culture.

Lymphoid Cell Cultures. Peyer's patch and spleen cell suspensions were prepared as described previously (1). Cultures contained 5×10^6 Peyer's patch cells from normal mice as well as irradiated Peyer's patch or spleen cells from mice fed or injected with erythrocytes as indicated in the text. In some instances irradiated Peyer's patch or spleen cells were depleted of T cells before culture by the methods described below. Culture methods followed the procedure described by Mishell and Dutton (7) for the study of mouse spleen cell suspensions. All cultures were incubated with 1 ml RPMI-1640 medium (Grand Island Biological Co., Grand Island, N. Y.) supplemented with 5% normal fetal bovine serum (FBS) (batch M 27904, Reheis Chemical Co., Chicago, Ill.) and ME (5×10^{-5} M) (Matheson, Coleman & Bell, Norwood, Ohio) (1). Cultures were fed daily with a nutritional mixture supplemented with the same FBS used in preparing the cultures (7).

Induction of Humoral Immune Responses. Cultures were stimulated with 3×10^6 TNP-SRBC, 3×10^6 TNP-CRBC, or no antigen, as indicated in the text. On the 4th day after culturing, the number of direct hemolytic plaque-forming cells (PFC) in each culture was assayed using a standard microscope slide assay (7). Cultures stimulated with TNP-SRBC or TNP-CRBC were assayed using lightly coupled TNP-HRBC, as well as HRBC and SRBC and/or CRBC. To analyze the specificity of the plaques, TNP_{19}-bovine serum albumin (BSA) was incorporated into each slide assay at a final concentration of 2×10^{-5} M. At this concentration there was no suppression of the formation of PFC directed against noncross-reacting determinants (SRBC) but greater than 95% inhibition of TNP-specific plaques. Data presented for TNP-specific PFC represent the number of PFC that were inhibited from developing on the slide assay in the presence of 2×10^{-5} M TNP_{19}-BSA. Background PFC observed in the absence of antigen stimulation of cultures has been subtracted from the reported data. In all experiments data have been expressed as the number of direct PFC/10^6 recovered viable cells. Cell viability was determined by trypan blue exclusion.

Irradiation of Lymphoid Cell Suspensions. Peyer's patch or spleen cell suspensions were irradiated in vitro with 1,200 R using a cobalt-60 source (1) as indicated in the text.

Rabbit Antimouse Brain (RAMB) Treatment of Peyer's Patch Cells. To deplete T cells, Peyer's patch cells were treated sequentially with RAMB antiserum (8) (generously supplied by Dr. Park Trefts, University of California, San Diego) and agarose absorbed guinea pig complement (C). Briefly, 5×10^7 viable Peyer's patch cells suspended in 1 ml of BSS were incubated with RAMB serum at a final dilution of 1:6 for 30 min at 37°C. The cells were then washed and incubated with 2 ml of a 1:7 dilution of guinea pig C for 30 min at 37°C. Cells were washed three times in BSS before being resuspended in complete medium. In our hands, treatment of spleen cells or Peyer's patch cells by this method completely abrogated the primary humoral immune response to SRBC in culture. Moreover, the ability of RAMB-treated spleen and Peyer's patch cells to support the induction of an anti-SRBC response in vitro was fully restored by the addition to culture of 1.5×10^6 irradiated (1,200 R) spleen cells from intravenous low dose SRBC-primed mice (4). Further characterization of this RAMB serum has been described elsewhere (8).

Results

Ability of Peyer's Patch Cells from SRBC-Fed Mice to Enhance the Anti-TNP Response of Peyer's Patch Cultures Stimulated with TNP-SRBC. The ability of irradiated Peyer's patch cells from SRBC-fed mice to enhance the anti-TNP response of Peyer's patch cultures stimulated with TNP-SRBC is shown in Table I. Addition of irradiated Peyer's patch cells from SRBC-fed mice to normal

TABLE I

Ability of Irradiated Peyer's Patch Cells from SRBC-Fed Mice to Enhance the Anti-TNP Response of Peyer's Patch Cultures Stimulated with TNP-SRBC

Contents of culture		Anti-TNP PFC/10^6 recovered viable cells	
Normal Peyer's patch cells (cells/culture)	Irradiated Peyer's patch cells SRBC-fed mice* (cells/culture)	Cultures stimulated with TNP-SRBC‡§	Cultures stimulated with TNP-CRBC‡
5×10^6	—	241	56
5×10^6	1×10^6	967	61
5×10^6	2.5×10^6	1,276	72
5×10^6	2.5×10^6 RAMB‖	332	Not assayed

* Peyer's patch cells from SRBC-fed mice were exposed to 1,200 R from a ^{60}Co source immediately before addition to culture. When cultured separately with antigen, these cells gave no response.

‡ Cultures were stimulated with 3×10^6 TNP-SRBC or 3×10^6 TNP-CRBC. Results represent the means of duplicate cultures.

§ The specific anti-SRBC response in normal Peyer's patch cultures stimulated with TNP-SRBC was 1,774 PFC/10^6. After the addition of 1×10^6 and 2.5×10^6 irradiated Peyer's patch cells from SRBC-fed mice, the specific anti-SRBC response rose to 5,669 and 6,049 PFC/10^6, respectively. RAMB treatment of irradiated Peyer's patch cells from SRBC-fed mice reduced the specific anti-SRBC response to 2,283 PFC/10^6.

‖ To deplete T cells, irradiated Peyer's patch cells from SRBC-fed mice were treated with RAMB serum and C, as described in the Materials and Methods.

Peyer's patch cultures consistently enhanced by four- to fivefold the specific anti-TNP (Table I) and anti-SRBC response of normal Peyer's patch cultures. Carrier priming was specific for determinants on the SRBC since enhancement of the anti-TNP response was not observed in parallel cultures stimulated with TNP-coupled CRBC (Table I). Anti-TNP responses were not enhanced further by adding greater numbers of irradiated Peyer's patch cells from SRBC-fed mice and in some experiments addition of 4×10^6 or 5×10^6 irradiated Peyer's patch cells from SRBC-fed mice reduced the magnitude of the anti-TNP response in normal Peyer's patch cultures. Depletion of T cells by RAMB treatment completely abrogated the carrier-priming effect mediated by Peyer's patch cells from SRBC-fed mice, indicating that helper function in these experiments was dependent on T cells.

Failure of Peyer's Patch Cells from CRBC-Fed Mice or Control Mice to Enhance the Anti-TNP Response of Peyer's Patch Cultures Stimulated with TNP-SRBC. Specificity of the induction of carrier priming of Peyer's patch T cells for helper function was characterized further by examining the ability of irradiated Peyer's patch cells from CRBC-fed mice to enhance the anti-TNP response of normal Peyer's patch cultures stimulated with TNP-SRBC (Table II). CRBC were chosen for these specificity experiments since CRBC were shown previously to exhibit minimal cross-reactivity at the T-cell level with SRBC (4, 9). As seen in Table II, addition of irradiated Peyer's patch cells from CRBC-fed mice to normal Peyer's patch cultures did not significantly enhance the anti-TNP response when cultures were stimulated with TNP-SRBC. The minimal increase in the anti-TNP responses sometimes observed (always less than

TABLE II

Failure of Irradiated Peyer's Patch Cells from CRBC-Fed Mice or Irradiated Normal Peyer's Patch Cells to Enhance the Anti-TNP Response of Peyer's Patch Cultures Stimulated with TNP-SRBC

Contents of culture*			Anti-TNP PFC/10^6 recovered viable cells§
Normal Peyer's patch cells (cells/culture)	Irradiated Peyer's patch cells CRBC-fed mice‡ (cells/culture)	Irradiated normal Peyer's patch cells‡ (cells/culture)	
5×10^6	—		334
5×10^6	1×10^6		394
5×10^6 ‖	2.5×10^6 ‖		536
5×10^6	2.5×10^6 RAMB¶		417
5×10^6		1×10^6	451
5×10^6		2.5×10^6	556
5×10^6		2.5×10^6 RAMB¶	512

* All cultures were stimulated with 3×10^6 TNP-coupled SRBC.

‡ Peyer's patch cells from CRBC-fed and normal mice were exposed to 1,200 R from a ^{60}Co source immediately before addition to culture. When cultured separately with antigen, these cells gave no response.

§ Results represent the means of duplicate cultures.

‖ Stimulation of parallel control cultures containing normal Peyer's patch cells (5×10^6) and irradiated Peyer's patch cells from CRBC-fed mice (2.5×10^6) with TNP-CRBC led to greater than 10-fold enhancement of the anti-TNP response.

¶ To deplete T cells, irradiated Peyer's patch cells from CRBC-fed mice and normal mice were treated with RAMB serum and C, as described in the Materials and Methods.

twofold) likely represents the slight degree of cross-reactivity (up to 5%) at the T-cell level between CRBC and SRBC (9). Stimulation of parallel control cultures containing normal Peyer's patch cells (5×10^6) and irradiated Peyer's patch cells from CRBC-fed mice (2.5×10^6) with TNP-CRBC resulted in a greater than 10-fold enhancement of the anti-TNP response. Finally, addition to normal Peyer's patch cell suspensions of irradiated Peyer's patch cells from normal control mice did not result in significant enhancement of the anti-TNP response in cultures stimulated with TNP-SRBC (Table II) or TNP-CRBC.

Ability of Irradiated Peyer's Patch Cells from SRBC-Fed Mice to Enhance the Anti-TNP Response of T-Cell-Depleted Peyer's Patch Cultures. Peyer's patch cells from normal mice were depleted of T cells by RAMB treatment (see Materials and Methods) and cultured together with irradiated Peyer's patch cells from SRBC-fed mice or irradiated Peyer's patch cells from normal control mice. As seen in Table III, addition of irradiated Peyer's patch cells from SRBC-fed mice to T-cell-depleted Peyer's patch cultures, restored the ability of these cultures to support the induction of an anti-TNP response when stimulated with TNP-SRBC (line 3). In contrast, addition of irradiated Peyer's patch cells from normal control mice did not restore the ability to induce an anti-TNP response in T-cell-depleted cultures (line 4). After depletion of T cells, the irradiated SRBC-fed Peyer's patch population no longer supported the induction of an anti-TNP response in T-cell-depleted cultures stimulated with TNP-SRBC (line 5),

TABLE III

Ability of Irradiated Peyer's Patch Cells from SRBC-Fed Mice to Enhance the Anti-TNP Response of T-Cell-Depleted Peyer's Patch Cultures

Peyer's patch cells cultured* (cells/culture)	Anti-TNP PFC/10^6 recovered viable cells‡
1. Normal Peyer's patch cells (5×10^6)	264
2. RAMB§-treated Peyer's patch cells (5×10^6)	<10
3. RAMB-treated Peyer's patch cells (5×10^6) + irradiated Peyer's patch cells, SRBC-fed mice (2.5×10^6)‖	248
4. RAMB-treated Peyer's patch cells (5×10^6) + irradiated Peyer's patch cells, normal control mice (2.5×10^6)‖	<10
5. RAMB-treated Peyer's patch cells (5×10^6) + RAMB-treated irradiated Peyer's patch cells, SRBC-fed mice (2.5×10^6)‖	<10

* All cultures were stimulated with 3×10^6 TNP-coupled SRBC.

‡ Results represent the means of duplicate cultures.

§ To deplete T cells, Peyer's patch cells were treated with RAMB serum and C, as described in the Materials and Methods.

‖ Peyer's patch cells from normal and SRBC-fed mice were exposed to 1,200 R from a ^{60}Co source immediately before addition to culture. When cultured separately with antigen, these cells gave no response.

indicating again that carrier priming for helper function in the SRBC-fed population was dependent on T cells. Although addition of irradiated Peyer's patch cells from SRBC-fed mice did support the induction of anti-TNP responses in T-cell-depleted cultures stimulated with TNP-SRBC, it is of note that the magnitude of these responses was consistently no greater than those observed in normal Peyer's patch cultures containing both B and T cells (compare line 3 and line 1).

Comparative Ability of Peyer's Patch Cells or Spleen Cells from Mice Immunized Intravenously or Intraperitoneally with SRBC to Enhance the Anti-TNP Response of Normal Peyer's Patch Cultures. The ability of irradiated Peyer's patch cells from mice injected intravenously or intraperitoneally with SRBC to enhance the anti-TNP response of normal Peyer's patch cultures stimulated with TNP-SRBC can be seen in Table IV. Addition of irradiated Peyer's patch cells from mice immunized either intravenously or intraperitoneally with SRBC did not enhance the anti-TNP response of Peyer's patch cultures stimulated with TNP-SRBC. In contrast, addition of irradiated spleen cells from mice primed intravenously or intraperitoneally with SRBC did lead to significant enhancement of the anti-TNP response of normal Peyer's patch cultures stimulated with TNP-SRBC (Table IV). In specificity controls, spleen cells from mice immunized intravenously or intraperitoneally with CRBC did not significantly enhance the anti-TNP response of normal Peyer's patch cultures stimulated with TNP-SRBC (data not shown).

Discussion

This study demonstrates that T cells in murine Peyer's patches can be specifically carrier primed for helper function in the induction of an antihapten

TABLE IV

Comparative Ability of Peyer's Patch Cells and Spleen Cells from Mice Immunized Intravenously or Intraperitoneally with SRBC to Enhance the Anti-TNP Response of Normal Peyer's Patch Cultures

	Contents of culture*			Anti-TNP PFC/10^6 recovered viable cells§
	Normal Peyer's patch cells (cells/culture)	Irradiated Peyer's patch cells SRBC-injected mice‡ (cells/culture)	Irradiated spleen cells SRBC-injected mice‡ (cells/culture)	
Intravenous injected Mice	5×10^6	—	—	331
	5×10^6	1×10^6	—	239
	5×10^6	2.5×10^6	—	306
	5×10^6	—	1×10^6	1,079
	5×10^6	—	2.5×10^6‖	1,643
Intraperitoneal injected Mice	5×10^6	1×10^6	—	272
	5×10^6	2.5×10^6	—	401
	5×10^6	—	1×10^6	1,347
	5×10^6	—	2.5×10^6‖	1,566

* All cultures were stimulated with 3×10^6 TNP-coupled SRBC.

‡ Peyer's patch or spleen cells from low dose SRBC-injected mice were exposed to 1,200 R from a ^{60}Co source immediately before addition to culture. When cultured separately with antigen these cells gave no response.

§ Results represent the means of duplicate cultures.

‖ Spleen cells from mice immunized intravenously or intraperitoneally with CRBC did not significantly enhance the anti-TNP response of Peyer's patch cultures stimulated with TNP-SRBC.

response by oral administration of antigen. Carrier priming for helper function was assessed by measuring the ability of irradiated Peyer's patch cells from mice fed carrier erythrocyte antigens to enhance an anti-TNP response in vitro when added to normal Peyer's patch cells cultured with TNP coupled to the erythrocyte used for feeding. Addition of irradiated Peyer's patch cells from SRBC-fed or CRBC-fed mice to Peyer's patch cultures from normal mice resulted in significant enhancement of the primary anti-TNP response when these cultures were stimulated with TNP coupled to the homologous carrier. Carrier priming was antigen specific. Peyer's patch cells from SRBC-fed mice enhanced the primary anti-TNP response of normal Peyer's patch cultures stimulated with TNP-SRBC but did not significantly enhance the anti-TNP response in parallel cultures stimulated with TNP-CRBC (Table I). Similarly, Peyer's patch cells from CRBC-fed mice enhanced the anti-TNP response of normal Peyer's patch cultures stimulated with TNP-CRBC, but did not enhance the anti-TNP response of parallel cultures stimulated with TNP-SRBC (Table II). Enhancement of the anti-TNP response was abrogated by depleting T cells from irradiated erythrocyte-fed Peyer's patch cells, indicating that carrier-specific helper function was dependent on T cells. Finally, carrier-specific priming of Peyer's patch T cells was observed only when antigen was administered by the oral route. Anti-TNP responses were enhanced when irradiated Peyer's patch cells from erythrocyte-

fed mice were added to normal cultures (Table I) but not when irradiated Peyer's patch cells from mice injected intravenously or intraperitoneally with erythrocytes were used (Table IV), although intravenous or intraperitoneal administration of erythrocytes resulted in significant carrier priming of T cells in the spleen (Table IV).

Irradiated Peyer's patch cells from SRBC-fed mice restored the ability to induce primary anti-TNP responses in T-cell-depleted Peyer's patch cultures (Table III), but the magnitude of the anti-TNP response was low when compared with normal cultures supplemented with irradiated Peyer's patch cells from SRBC-fed mice (Tables I and III). This result could be interpreted in several ways. The difference could be explained simply if T-cell depletion (i.e., RAMB serum and C) nonspecifically damaged TNP-specific B cells. In this respect however, primary anti-SRBC responses in RAMB-treated Peyer's patch and spleen cultures were restored to normal levels by the addition of irradiated low dose primed spleen cells, suggesting that RAMB treatment did not result in significant B-cell damage. Alternately, the difference in the magnitude of the anti-TNP response between normal and T-cell-depleted cultures supplemented with irradiated carrier-primed T cells from SRBC-fed mice could be interpreted to suggest a T-cell-T-cell interaction in which irradiated carrier-primed T cells from SRBC-fed mice enhanced the induction of additional carrier-primed T cells in the normal cultures stimulated with the hapten-carrier conjugate.

Although T cells in Peyer's patches can be carrier primed for helper function by antigen feeding as described in this report, B cells in Peyer's patches could not be induced to humoral antibody synthesis by feeding protein (3) or erythrocyte antigens (reference 2 and M. Kagnoff, unpublished observations). Several possibilities might be considered when attempting to explain the failure to induce B cells in Peyer's patches to humoral antibody synthesis in vivo. First, the possibility that B cells are not induced due to the failure of antigen to gain access to the Peyer's patch antigen-sensitive cell compartment can be excluded since this study demonstrates that T cells in Peyer's patches can be specifically carrier primed for helper function by feeding erythrocytes. The finding that at least some antigenic determinants on the erythrocyte interact with antigen-sensitive cells in Peyer's patches is not surprising since previous studies showed that specialized epithelial cells overlying Peyer's patches (10, 11) took up India ink (10, 12) and ferritin particles (12) from the intestinal lumen and these particles subsequently appeared within Peyer's patches (12). Although antigenic determinants gain access to Peyer's patch cells from the intesinal lumen, the possibility that the effective antigen concentration to which the Peyer's patch B cells are exposed is sufficiently low to limit their induction has not been excluded. A low effective antigen concentration might result because of an inability to localize antigen in Peyer's patches (3) or because of a lack of appropriate antigen processing or antigen presentation to the B cell, perhaps due to a deficiency of macrophage-like cells. Second, the possibility that B cells in Peyer's patches are paralyzed rather than induced to humoral antibody synthesis after interaction with antigen must be considered. Peyer's patches are naturally deficient in the cooperating accessory adherent cell type(s) required in vitro for B-cell induction (1). Evidence supporting the concept that deficient

accessory adherent cell function might result in immunologic unresponsiveness when antigen is presented directly to populations of B and T cells has been recently discussed (13). Moreover, when viewed in light of one 2-signal model for B-cell induction and paralysis (14, 15) Peyer's patch B cells might be predicted to enter a paralytic pathway since generation of an effective second signal for B-cell induction to most thymus-dependent antigens likely requires the presence of both cooperating T cells and macrophage-like accessory adherent cells (16, 17). However, preliminary in vivo studies in our laboratory showed that SRBC feeding did not reduce the ability to subsequently induce anti-SRBC responses in Peyer's patch cultures and in vitro incubation of Peyer's patch cells with SRBC before the addition of ME did not abolish the anti-SRBC response (M. Kagnoff, unpublished observations). These findings suggest that B cells in Peyer's patches are not paralyzed by feeding erythrocytes. Third, the observation that T cells in Peyer's patches can be specifically carrier-antigen primed for helper function in the absence of B-cell induction to humoral antibody synthesis suggests the possibility that the requirement for cooperating cells for the induction of B cells to humoral antibody synthesis differs at least quantitatively from the requirement for cooperating cells for the priming of T cells for helper function. In this respect, T-cell priming appears to be far less dependent on the presence of macrophage-like accessory adherent cells. Although murine Peyer's patches are naturally deficient in the cooperating accessory adherent cell type(s) required for the induction of humoral antibody synthesis in vitro (1), humoral antibody synthesis to erythrocyte antigens can be induced in vitro when Peyer's patch cultures are supplemented with syngeneic PAC or ME (1). Peyer's patches contain dendritic reticular cells and macrophage-like cells on histological examination (18, 19), however these cells appear to differ from the cooperating accessory adherent cells required for B-cell induction in vitro. Macrophages derived from bone marrow also were unable to support the induction of humoral immune responses in vitro (20). Recent evidence indicates that Peyer's patches are deficient also in the effector cell type required to kill antibody-sensitized erythrocyte target cells by an antibody-dependent cell-mediated cytotoxic (ADCC) mechanism,[2] but there is little evidence to support the possibility that the same accessory adherent cell type is required for both B-cell induction and target cell killing by an ADCC mechanism (21).

Peyer's patches in rabbits (22) and mice (23) have been reported to contain B cells which populate the intestinal lamina propria and produce IgA. Although B cells from rabbit Peyer's patches appear to home directly to the intestinal lamina propria, studies on mice suggest that the migratory pattern of Peyer's patch cells may be more complex (1, 23, 24). The physiological role of our finding that Peyer's patch T cells can be specifically primed for helper function may be clarified, at least in part, by characterizing further the homing properties and the functional heterogeneity of T cells in Peyer's patches. It is tempting to speculate however that Peyer's patch T cells primed by gut antigens provide a population of memory T cells which migrate to other lymphoid sites, including

[2] Kagnoff, M. F., and S. Campbell. 1975. Antibody-dependent cell-mediated cytotoxicity: comparative ability of murine Peyer's patch and spleen cells to lyse lipopolysaccharide-coated and uncoated erythrocytes. *Gastroenterology*. In press.

perhaps the intestinal lamina propria, and serve an important function in the host's interaction with intraluminal gut antigens. Finally, it is important to note that T cells mediating helper function are radiation resistant (25, 26) while T cells which suppress the B-cell response appear, at least in some stages of differentiation, to be radiation sensitive (27, 28). Since T-cell helper function was assayed using irradiated Peyer's patch cells exclusively, our results would not reveal a radiation-sensitive suppressor T-cell population. It is impossible to state with certainty therefore that antigen feeding leads to the generation of T helper cells only.

Summary

Peyer's patch T cells may serve an important role in the interaction of the host with intraluminal gut antigens. Studies presented in this paper demonstrate that T cells in murine Peyer's patches can be carrier primed for helper function in the induction of an antihapten response by feeding antigen. Carrier priming was assessed by measuring the ability of Peyer's patch cells from mice fed heterologous erythrocytes to enhance an antitrinitrophenyl (TNP) response in vitro when added to normal Peyer's patch cells cultured with TNP coupled to the erythrocyte used for feeding. Priming of T helper cells in Peyer's patches was antigen specific and occurred when erythrocytes were administered orally but not when erythrocytes were injected intravenously or intraperitoneally. Murine Peyer's patches are naturally deficient in a cooperating accessory adherent cell type(s) required for B-cell induction to humoral antibody synthesis in vitro and antigen feeding does not result in significant induction of Peyer's patch B cells to humoral antibody synthesis in vivo. Since Peyer's patch T cells can be carrier-antigen primed for helper funtion in the absence of B-cell induction to humoral antibody synthesis, these studies may indicate that T-cell priming is less dependent than B-cell induction on cooperating accessory adherent cells.

The author is grateful to Ms. B. Claudy for expert technical assistance. Doctors Melvin Cohn, Park Trefts, and James Watson are acknowledged for their helpful comments on the manuscript.

Received for publication 23 July 1975.

References

1. Kagnoff, M. F., and S. Campbell. 1974. Functional characteristics of Peyer's patch lymphoid cells. I. Induction of humoral antibody and cell-mediated allograft reactions. *J. Exp. Med.* **139**:398.
2. Henry, C., W. P. Faulk, L. Kuhn, J. N. Yoffey, and H. H. Fudenberg. 1970. Peyer's patches: immunologic studies. *J. Exp. Med.* **131**:1200.
3. Bienenstock, J., and J. Dolezel. 1971. Peyer's patches: lack of specific antibody-containing cells after oral and parenteral immunization. *J. Immunol.* **106**:938.
4. Kettman, J., and R. W. Dutton. 1970. An *in vitro* primary immune response to 2,4,6-trinitrophenyl substituted erythrocytes: response against carrier and hapten. *J. Immunol.* **104**:1558.
5. Rittenberg, M. B., and K. L. Pratt. 1969. Antitrinitrophenyl (TNP) plaque assay. Primary response of Balb/c mice to soluble and particulate immunogen. *Proc. Soc. Exp. Biol. Med.* **132**:575.

6. Falkoff, R., and J. Kettman. 1972. Differential stimulation of precursor cells and carrier-specific thymus-derived cell activity in the *in vivo* response to heterologous erythrocytes in mice. *J. Immunol.* **108**:54.
7. Mishell, R. I., and R. W. Dutton. 1967. Immunization of dissociated spleen cell cultures from normal mice. *J. Exp. Med.* **126**:423.
8. Linthicum, D. S., S. Sell, R. M. Wagner, and P. Trefts. 1974. Scanning immunoelectron microscopy of mouse B and T lymphocytes. *Nature (Lond.).* **252**:173.
9. Hoffman, M., and J. W. Kappler. 1973. Regulation of the immune response. II. Qualitative and quantitative differences between thymus- and bone marrow-derived lymphocytes in the recognition of antigen. *J. Exp. Med.* **137**:721.
10. Bockman, D. E., and M. D. Cooper. 1973. Pinocytosis by epithelium associated with lymphoid follicles in the bursa of fabricius, appendix and Peyer's patches. An electron microscopic study. *Am. J. Anat.* **136**:455.
11. Owen, R. L., and A. L. Jones. 1974. Epithelial cell specialization within human Peyer's patches: an ultrastructural study of intestinal lymphoid follicles. *Gastroenterology.* **66**:189.
12. Joel, D. D., B. Sordat, M. W. Hess, and H. Cottier. 1970. Uptake and retention of particles from the intestine by Peyer's patches in mice. *Experientia (Basel).* **26**:694. (Abstr.)
13. Miller, R. G. 1974. The role of accessory (A) cells in specific immunological unresponsiveness. *In* Progress in Immunology II. L. Brent and J. Holborow, editors. American Elsevier Publishing Co., Inc., New York. **3**:229.
14. Bretscher, P., and M. Cohn. 1970. A theory of self-nonself discrimination. Paralysis and induction involve the recognition of one and two determinants on an antigen, respectively. *Science (Wash. D. C.)* **169**:1042.
15. Bretscher, P. 1972. The control of humoral and associative antibody synthesis. *Transplant. Rev.* **11**:217.
16. Feldmann, M. 1972. Cell interactions in the immune response in vitro. V. Specific collaboration via complexes of antigen and thymus-derived cell immunoglobulin. *J. Exp. Med.* **136**:737.
17. Schrader, J. W. 1973. Mechanism of activation of the bone marrow-derived lymphocyte. III. A distinction between a macrophage-produced triggering signal and the amplifying effect on triggered B lymphocytes of allogeneic interactions. *J. Exp. Med.* **138**:1466.
18. Sobhon, P. 1971. The light and the electron microscopic studies of Peyer's patches in non germ-free adult mice. *J. Morphol.* **135**:457.
19. Waksman, B. H. 1973. The homing pattern of thymus-derived lymphocytes in calf and neonatal mouse Peyer's patches. *J. Immunol.* **111**:878.
20. Watson, J., M. Thoman, P. Ralph, and E. Trenkner. 1974. The role of humoral factors in the initiation of *in vitro* primary immune responses. IV. Are macrophages the adherent cell type required for cell cooperation. *J. Immunol.* **112**:1873.
21. Scornik, J. C., and H. Cosenza. 1974. Antibody-dependent cell-mediated cytotoxicity. III. Two functionally different effector cells. *J. Immunol.* **113**:1527.
22. Craig, S. W., and J. J. Cebra. 1971. Peyer's patches: an enriched source of precursors for IgA-producing immunocytes in the rabbit. *J. Exp. Med.* **134**:188.
23. Guy-Grand, D., C. Griscelli, and P. Vassalli. 1974. The gut-associated lymphoid system: nature and properties of the large dividing cells. *Eur. J. Immunol.* **4**:435.
24. McWilliams, M., J. M. Phillips-Quagliata, and M. E. Lamm. 1975. Characteristics of mesenteric lymph node cells homing to gut-associated lymphoid tissue in syngeneic mice. *J. Immunol.* **115**:54.
25. Kettman, J., and R. W. Dutton. 1971. Radioresistance of the enhancing effect of cells

from carrier-immunized mice in an *in vitro* primary immune response. *Proc. Natl. Acad. Sci. U. S. A.* **68**:699.

26. Hamaoka, T., D. H. Katz, and B. Benacerraf. 1972. Radioresistance of carrier-specific helper thymus-derived lymphocytes in mice. *Proc. Natl. Acad. Sci. U. S. A.* **69**:3453.
27. Dutton, R. W. 1972. Inhibitory and stimulatory effects of concanavalin A on the response of mouse spleen cell suspensions to antigen. I. Characterization of the inhibitory cell activity. *J. Exp. Med.* **136**:1445.
28. Dutton, R. W. 1975. Suppressor T cells. *Transplant. Rev.* **26**: in press.