Brief Definitive Report

PREFERENTIAL INDUCTION OF POLYCLONAL IgA SECRETION BY MURINE PEYER'S PATCH DENDRITIC CELL–T CELL MIXTURES

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Our laboratory has recently demonstrated that enzymatically-dissociated Peyer's patches (PP) possess all the lymphoreticular cell populations necessary for induction of thymus-dependent (TD) antigen-specific IgM and IgA responses (1). Prior oral administration of TD antigen markedly augmented this IgA response, and resulted in induction of antigen-specific T helper cells in PP (1).

In contrast, we and others have had little success in generating significant polyclonal IgA responses by PP cells using conventional T and/or B cell mitogens (2–5). This failure might be due to: (*a*) inadequacy of helper cells (T cells and/or accessory cells); (*b*) excess suppressor T cells; or (*c*) inability of precursor B cells to respond to a polyclonal stimulus.

We have recently modified our methods for enrichment of the Peyer's patch dendritic cell (PP DC) (6) to provide PP or spleen populations containing predominantly DC and T cells of the helper phenotype. We were intrigued by the possibility that these cell mixtures might provide an enriched source of suitably activated helper T cells for selective enhancement of IgA responses. We report here the successful induction of significant polyclonal IgA secretion (both by PP and spleen B cells), which is dependent upon the interaction of these B cells with dendritic cell–T cell mixtures derived from murine PP. This observation of polyclonal induction of IgA synthesis in murine B cells should facilitate elucidation of the roles of accessory cells and regulatory T cells in the induction of IgA isotype responses.

Materials and Methods

Mice. C3H/HeJ and C3H/HeN mice were obtained from the Immunocompromised Mouse Core Facility, Tumor Institute, University of Alabama in Birmingham and from the Frederick Cancer Research Center, Frederick, MD. Mice were generally used at 8 wk of age.

Preparation of Cell Mixtures from PP and Spleen Enriched in DC and Lyt-1$^+$T Cells. PP and spleen cells from C3H/HeJ or C3H/HeN mice were obtained by enzymatic dissocia-

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tion with Dispase as previously described (5, 6). Unfractionated cells were treated with sodium metaperiodate (6), suspended in complete media [RPMI 1640 (M.A. Bioproducts, Walkersville, MD); gentamycin, 20 μg/ml (Schering Corporation, Kenilworth, NJ); L-glutamine, 2 mM (Grand Island Biological Co., Grand Island, NY); 5% fetal calf serum (FCS) (Flow Labs, McLean, VA), 2-mercaptoethanol, 5×10^{-5} M (Bio-Rad Laboratories, Richmond, CA); and indomethacin, 1 μg/ml (Sigma Chemical Company, St. Louis, MO)] and cultured at 10×10^6 cells/750 μl in 24-well plates. During overnight incubation, large cell aggregates (clusters) formed, which were separated from single cells by centrifugation on a continuous density gradient of BSA ($\rho = 1.008 \rightarrow 1.030$) (6). The isolated cell clusters were washed twice in RPMI 1640, resuspended in complete media, and cultured in 24-well plates at $1-2 \times 10^6$ cells/ml. These cells from the cluster fraction were cultured 72 h to permit T cell proliferation and removal (by adherence) of residual macrophages. The nonadherent cluster-derived cells were then harvested and separated into high and low density populations on a dense BSA cushion ($\rho = 1.078$). A low density population derived from the original clusters (referred to as DC-T for simplicity) was thus obtained and further characterized (see Results) by immunofluorescence with anti–Thy-1.2 (Becton-Dickinson, Sunnyvale, CA), anti–Lyt-1 (clone 53-7.313 from American Type Culture Collection), anti–Lyt-2 (clone 53-6.72 from ATCC), anti-IgA and anti-IgM (kindly provided by Dr. John F. Kearney, University of Alabama in Birmingham). Cytotoxicity was determined with anti-Ia^k (clone 11-5.2 from the Salk Institute) or anti–Thy-1.2 and complement (Low Tox M Rabbit Complement; Accurate Scientific, Westbury, NY).

Preparation of DC-T from Isolated PP DC and PP T Cells. PP DC were isolated from C3H/HeN mice as previously described (6) except that B cells were eliminated from unfractionated PP by panning on anti-mouse immunoglobulin-coated plates (7) before the isolation procedure. T cells were obtained by treatment of unfractionated PP with anti-Ia^k and C and anti-Ig (Meloy Labs, Inc., Springfield, VA) and C. The PP DC and T populations were then co-cultured and clusters separated and handled as described above. Experiments with isolated DC and T cells were performed with C3H/HeN strain mice rather than the high IgA responder C3H/HeJ strain used in the preceding experiments because of a shortage of the C3H/HeJ mice from our facility. Variability in the inductive capability has been noted in multiple experiments with the C3H/HeN strain as opposed to the uniformly potent stimulus seen with C3H/HeJ mice. Experiments are currently in progress to determine the mechanism of this differential capability between strains that are congenic except for the *Lps* gene.

Preparation of B Cells. Spleen and PP B cell populations were obtained by panning (7) enzymatically treated PP and conventionally prepared spleen cell suspensions on petri dishes coated with the IgG fraction of a goat anti-mouse immunoglobulin antisera.

Co-cultivation of Low Density Cluster Derived Cells and B Cells. Low density cluster derived cells (DC-T) from spleen or PP (generally 5×10^5 cells in 0.25 ml) were co-cultured with either spleen or PP B cells (1×10^6 cells in 0.5 ml) in complete media in 24- or 48-well culture dishes (37°C, Mishell-Dutton gas mixture). After 7 d, the culture supernatants were harvested and assayed for IgA and IgM using previously described isotype-specific radioimmunoassays (RIA) (5). IgAκ and IgAλ were detected using similar RIA in which wells were coated with either anti-κ or anti-λ (rabbit antisera diluted 1:100 obtained from Litton Bionetics, Kensington, MD) and using TEPC-15 (IgAκ) or MOPC-315 (IgAλ), respectively, as standards.

Results

T or B Cell Mitogens Are Unable to Induce Polyclonal IgA Secretion. Optimal culture conditions for both IgA and IgM synthesis by unfractionated PP or spleen cells from C3H/HeJ or C3H/HeN mice required 2-mercaptoethanol (5×10^{-5} M) in the culture media; this consistently resulted in enhancement of baseline immunoglobulin synthesis (Table I). However, we were unable to obtain significant enhancement of IgA secretion by unfractionated PP cells cultured

TABLE I

IgA Production by Unfractionated PP Cultures (ng/10^6 Cells)

Cells*	No mitogen	Con A‡ 2.0 μg	Con A 1.0 μg	PWM	Bu LPS 100 μg	M1g 100 μg
C3H/HeN						
(without 2-ME)	200	740	700	120	320	1,160
(with 2-ME§)	1,320	1,360	1,360	920	1,360	1,700
C3H/HeJ (with 2-ME)	2,100	2,900	2,400	2,080	3,200	2,200

* Enzymatically dissociated unfractionated PP cells were cultured at 2.5×10^6 cells/ml in 24-well culture dishes.
‡ Mitogens added to give the quantity indicated in each culture.
§ 2-Mercaptoethanol added to culture media to yield a final concentration of 5×10^{-5} M.

TABLE II

*IgA and IgM Secretion by PP or Spleen B Cells (ng/Culture)**

	PP B cells only		PP DC-T + PP B		Spleen DC-T + PP B	
	IgA	IgM	IgA	IgM	IgA	IgM
Exp. 1	363	375	8,812	3,150	—	—
Exp. 2	75	890	2,648	1,180	82	1,716
Exp. 3	75	412	17,250	4,012	233	5,520
Exp. 4	160	200	4,049	460	—	—
Exp. 5	128	468	5,809	813	—	—
Exp. 6	48	94	3,115	1,084	66	830
	Spleen B cells only		PP DC-T + spleen B		Spleen DC-T + spleen B	
Exp. 1	60	863	5,200	3,338	—	—
Exp. 2	30	3,068	2,452	5,317	40	4,500
Exp. 3	50	682	7,725	5,070	82	5,842

* B cell cultures contained 10^6 cells (in 0.5 ml medium) obtained by panning on plastic dishes with anti-mouse Ig. 5×10^5 DC-T were added in 0.25-ml volumes to appropriate cultures. C3H/HeJ strain was used as source of B cells and DC-T in these experiments.

with mercaptoethanol and the following mitogens: Con A, pokeweed mitogen, butanol-extracted lipopolysaccharide (LPS) or M1*g* (a streptococcal carbohydrate B cell mitogen) (Table I). Therefore, we sought to determine whether populations enriched in DC and T cells could provide an inductive stimulus.

Characterization of Low Density Cells Derived from PP Clusters. Immunofluorescence staining of the cluster-derived low density cells demonstrated surface staining for IgA in <0.5% of the cells (3 out of 1,050 cells). With cytoplasmic immunofluorescence staining we observe that <0.4% (6 out of 1,400) were positive for IgA and <1.2% (9 out of 700) were positive for IgM. By phase contrast light microscopy ~30–40% of this cell population exhibited dendritic morphology. In two experiments, anti-Ia^k plus complement killed 40% of the low density cell population. Immunofluorescence staining demonstrated that 45–55% of the cells bore Thy-1.2, and in two experiments with C3H/HeN mice >99% of the T cells were Lyt-1^+ and <1% were Lyt-2^+, clearly suggesting that the T cell population was largely of the inducer-helper phenotype.

Induction of Polyclonal IgA Secretion by DC-T Obtained From PP. The levels of IgA and IgM secreted by PP or spleen B cell cultures are shown in Table II. The DC-T derived from PP induced increases of 25–230-fold in IgA secretion by PP B cells, with 10-fold increase in IgM. This inductive capability was limited

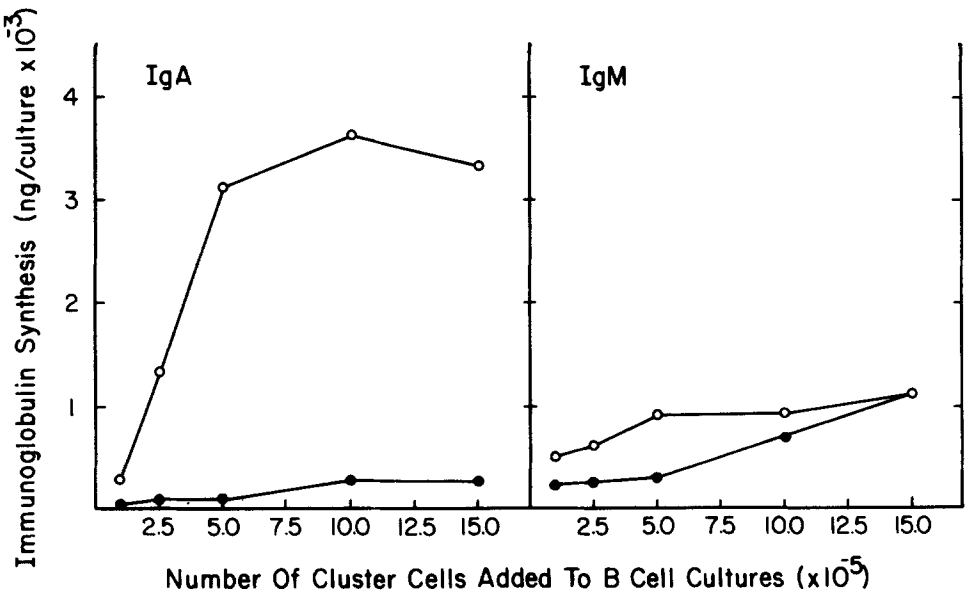

FIGURE 1. Dose response of immunoglobulin secretion induced by DC-T. Increasing numbers of PP (○) or spleen (●) DC-T were added (in 0.25-ml volumes) to 0.5-ml cultures containing 1×10^6 PP B cells, and incubated for 7 d. The IgA and IgM levels in the culture supernatants were determined by RIA. Supernatants were assayed at three different dilutions, each in duplicate. C3H/HeJ strain utilized.

to PP DC-T, since three-fold increases in IgA production occurred with spleen DC-T. Spleen B cells could also be induced to secrete significant quantities of IgA when PP DC-T were added. IgA production was increased 80–150-fold, while the increase in IgM was less than 10-fold. Again, the spleen DC-T did not enhance IgA production.

Dose response studies demonstrated a dose-dependent increase in IgA secretion to a plateau level when PP DC-T were added to PP B cells (Fig. 1). In contrast, enhancement of IgM synthesis was minimal at the lowest dose of DC-T and did not significantly increase when increasing numbers of DC-T were added. When increasing numbers of spleen DC-T were added to PP B cell cultures, there was no significant enhancement of IgA or IgM secretion.

In order to further define the nature of the cells responsible for the inductive stimulus, we utilized the low density cluster derived population obtained from the co-culture of isolated PP DC and PP T cell populations. Results (Table III) demonstrate that this combination is capable of significant augmentation of IgA secretion (equal or greater than DC-T from unfractionated PP) with minimal induction of IgM secretion. Co-cultures of B cells with isolated periodate-treated T cells (up to 1×10^6 cells/culture) or PP DC (up to 2×10^5 cells/cultures) in three experiments never induced more than a threefold increase in IgA secretion. These data confirm that the DC and T cells within the cluster-derived populations described above are the critical elements, but do not allow us to assess the relative importance of each population. In other studies now in progress we have evidence that both the T cell and the DC must come from PP to achieve optimal augmentation.

TABLE III

Induction of IgA and IgM by DC-T from Isolated PP DC and T Cells

	PP B cells only*	PP B cells + DC-T from isolated DC and T	PP B cells + DC-T from B cell depleted PP‡
IgA			
Exp. 1§	205	2,600	1,250
Exp. 2‖	75	2,750	2,100
IgM			
Exp. 1	300	1,050	750
Exp. 2	625	2,100	1,550

* B cells were cultured at 1×10^6 cells/ml/culture. C3H/HeN strain mice were utilized as cell source for B cells and DC-T.

‡ Variability in background IgA secretion by DC-T obtained from unfractionated C3H/HeN PP necessitated elimination of B cells by panning before sodium periodate treatment.

§ 3.75×10^5 DC-T added to 1×10^6 B cells in total volume of 1 ml.

‖ 2.75×10^5 DC-T added to 1×10^6 B cells in total volume of 1 ml.

Additional evidence that B cells contaminating the PP DC-T were not the source of the secreted IgA was provided by assay of supernatants from DC-T cultured alone; generally <200 ng IgA/culture (or <10% of the total IgA secreted by B cells induced by DC-T) was detectable. To ensure that the response was polyclonal in nature, culture supernatants were assayed for IgAκ and IgAλ using solid phase RIA. Results demonstrated that both IgAκ and IgAλ were produced, confirming that the induced IgA response was indeed polyclonal.

Discussion

The results of this study clearly demonstrate that polyclonal IgA secretion by murine B cells is inducible in vitro, and that PP contain cell types that preferentially induce IgA secretion. This inductive stimulus can activate B cells from either PP or spleen to secrete IgA. Although this induction is dependent on the interaction of DC and Lyt-1^+ T cells from PP, our studies do not yet permit conclusions concerning the relative importance of the DC, T cell, or T cell–derived soluble factors. Very recent studies have demonstrated that DC-T from spleen can provide helper factors for antigen-specific B cell responses of the IgM isotype (8). The specificity of the responses for IgA secretion in the present study suggests that nonspecific T cell–derived factors are not critical, but an isotype-specific helper factor similar to that recently described in human T-T hybridomas (9) may be involved.

Recent studies have shown that certain PP T cell clones can induce B cells to switch from surface IgM to surface IgA (sIgA$^+$) (4). It has been suggested that these T cells are important for the development of IgA precursor B cells in the PP. Interestingly, the switched sIgA$^+$ B cell cannot be induced by LPS to secrete IgA. The relationship between these T switch cells and DC-T capable of induction of B cell differentiation to IgA secretion remains to be elucidated.

Summary

Polyclonal IgA secretion is inducible in murine B cells when DC-T from Peyer's patches (PP) provide the inducing stimulus. PP DC-T, which are composed predominantly of dendritic cells and Lyt-1^+ T cells, are capable of dramatic

augmentation of IgA secretion by PP or spleen B cells with minimal induction of IgM secretion. DC-T from spleen, however, are incapable of augmenting IgA secretion by either PP or spleen B cells. The level of IgA secretion is dependent upon the dose of DC-T providing the inducing stimulus and reaches a plateau with DC-T:B ratios of less than 1:1. This system for preferential induction of IgA responses should permit elucidation of cellular mechanisms involved in regulation of IgA secretion.

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