ISOTYPE SPECIFICITY OF HELPER T CELL CLONES

Peyer's Patch Th Cells Preferentially Collaborate with Mature IgA B Cells for IgA Responses

By HIROSHI KIYONO, MAX D. COOPER, JOHN F. KEARNEY,
LISA M. MOSTELLER, SUZANNE M. MICHALEK, WILLIAM J. KOOPMAN,
AND JERRY R. McGHEE

From the Departments of Microbiology, Pathology, Pediatrics, and Medicine, The Institute of Dental Research, The Comprehensive Cancer Center, The Cellular Immunobiology Unit, and Birmingham Veteran's Administration Hospital, The University of Alabama in Birmingham, University Station, Birmingham, Alabama 35294

Two major types of T cells that deliver help (Th)[1] for B cell responses to thymic-dependent (TD) antigens have been described. The classic Th cell responds to carrier antigenic determinants and generates help for B cell responses to haptenic determinants (1–5). Induction of carrier-specific Th cells requires histocompatible accessory cells (6–8). MHC requirements for Th-B cell interactions, however, have been controversial (9–16). Studies with cloned murine Th cells (17–19) have revealed that activation of small, resting B cells requires the help of MHC-restricted, antigen-specific cells (20, 21). Once activated, B cells can be induced to divide and mature under the influence of T cell–derived B cell growth and differentiation factors. It has also been shown that activation of Lyb-5$^-$ B cells requires MHC restriction and hapten-carrier linkage for Th-B cell interaction (22, 23), while activation of Lyb-5$^+$ B cells is MHC unrestricted and can be triggered by hapten and carrier on separate molecules (22, 23).

Another class of Th cells appears to depend upon B cell immunoglobulin (Ig) determinants for induction. A subpopulation of helper T cells that recognizes Ig (Th$_{Ig}$) and normally acts in synergy with carrier-specific Th cells, cannot be detected in anti-μ suppressed mice that lack Ig$^+$ B cells (24, 25). Th$_{Ig}$ cells may express two receptors, one for idiotype and the other for antigen (26). These T cells interact with B cells via idiotype recognition and require antigen stimulation before collaboration with B cells. Induction of Th$_{Ig}$ cells in an *Igh*-rectricted environment leads to efficient help of B cells bearing the matching *Igh*-linked product (27). Antigen-activated B cells appears to be responsible for induction

[1] *Abbreviations used in this paper*: C, complement; Con A, concanavalin A; FACS, fluorescence-activated cell sorter; FCS, fetal calf serum; FITC, fluorescein-isothiocyanate; GALT, gut-associated lymphoreticular tissue; HBSS, Hanks' balanced salt solution; MEM, minimum essential medium; PFC, plaque-forming cells; PP, Peyer's patch; SRBC, sheep erythrocytes, sIg, surface immunoglobulin, TCGF, T cell growth factor; TC medium, growth medium for T cell clones; TD, thymic dependent; Th, T helper.

of Th_{Ig} cells (28). The cumulative evidence leads to the conclusion that subsets of Th cells can support either idiotype- (29, 30), allotype- (31), or isotype- (28, 32–34) specific B cell responses to TD antigens.

A major inductive site for murine IgA responses is the gut-associated lympho-reticular tissue (GALT), e.g., Peyer's patches (PP), which contain significant numbers of IgA-committed B cells and T cells that support IgA responses (35). We have isolated and characterized several T cell clones from murine PP that exhibit antigen-specific helper activity predominantly for IgA isotype responses (PP Th A; reference 36). These PP Th A clones supported higher IgA responses in cultures of B cells from PP than from spleen. Since PP contain an abundance of surface IgA-positive ($sIgA^+$) B cells, one explanation for higher responses in PP B cell cultures could be that PP Th A cells act directly on the postswitched $sIgA^+$ B cell subpopulation, perhaps via soluble differentiation factors (37). Alternatively, the PP Th A cells could induce B cell isotype switching, and recent work with mitogen-activated PP T cell clones supports this possibility (38, 39). In order to distiguish an isotype switch mechanism from preferential induction of $sIgA^+$ B cells, we have examined the B cells that collaborate with PP Th A cell clones for IgA responses.

Materials and Methods

Mice. C3H/HeJ (The Jackson Laboratory, Bar Harbor, ME) and C3H/HeN (National Institutes of Health, Bethesda, MD) mice were bred and maintained in laminar flow units in the University of Alabama in Birmingham Cancer Center, a facility designed for immunocompromised mice. All mice used in these studies were 7–12 wk of age, except for the newborn and young mice used in the study of IgA B cell ontogeny.

Culture Conditions for PP Th A Clones. Helper T cell clones used here were derived from PP of C3H/HeJ mice, as previously described (36). Briefly, C3H/HeJ mice were given sheep erythrocytes (SRBC; Colorado Serum Co., Denver, CO) by gastric intubation (daily for two consecutive days), their PP aseptically removed 1 wk later and cells dissociated to single cell suspensions with the enzyme Dispase (35). T cells were purified, cultured in RPMI 1640 (Grand Island Biological Co., [Gibco], Grand Island, NY) containing 2 mM L-glutamine, penicillin (100 U/ml), streptomycin (100 μg/ml), gentamicin (50 μg/ml), and 15 mM HEPES (Gibco) (incomplete TC medium), 10% fetal calf serum (FCS; Flow Laboratories, McLean, VA) and 2-mercaptoethanol (2-ME; 5×10^{-5} M) (complete TC medium). SRBC and T cell growth factor (TCGF) were added and T cells were cultured in an atmosphere of 7% O_2, 10% CO_2, and 83% N_2. Wells exhibiting cell growth after 2–3 wk were cloned by limiting dilution (17, 36). Individual clones were assessed for helper activity in B cell cultures immunized with SRBC. A total of 21 clones were selected which supported either mainly IgA with some IgM (9 clones) or high IgA and low IgM, IgG_1, IgG_2, and IgG_3 (12 clones) anti-SRBC plaque-forming cell (PFC) responses (36).

Feeder Cells. Single cell suspensions of spleens from C3H/HeN mice were treated with a cocktail of monoclonal antibodies (anti-mouse Thy-1.2 [Clone HO-13-4], anti–Lyt-1 [Clone 53-7.313] and -Lyt 2 [Clone 53-6.72] antibodies) for 30 min at 4°C, followed by incubation with anti-rat IgG and rabbit complement (C) for 30 min at 37°C. Cells were washed twice by centrifugation and the pelleted cells were irradiated (3,000 rads). Cells were washed and resuspended in complete TC medium and $1–2 \times 10^6$ cells were added to macroculture plates (Linbro Chemical Co., Hamden, CT). Cultures were incubated for 2–3 h at 37°C in 5% CO_2 in air and nonadherent cells were removed by vigorous pipetting and addition and removal of media. In other studies, peritoneal cells were removed by flushing the exposed abdominal cavities of C3H/HeN mice with 10–12 ml of incomplete

TC medium; cells were washed twice before irradiation (3,000 rads). Cells were added $(1-2 \times 10^5)$ to cultures and nonadherent cells removed as described above.

TCGF Production and Assay. T cell growth factor (TCGF) was prepared from Con A–induced spleen cell culture supernatants as described previously (36). TCGF was also obtained from the EL-4 thymoma cell line (40). EL-4 cells were cultured $(1 \times 10^6/\text{ml})$ in incomplete TC medium with 1% FCS in the presence of 10 ng of phorbol-12-myristate-13-acetate (PMA) for 40 h as described (40). Culture supernatants were obtained following centrifugation, filter sterilized, and TCGF activity assessed.

The TCGF-dependent helper T cell clone (HT-2) was used for titration of TCGF (17, 41). HT-2 cells were cultured in triplicate in flat-bottomed microplate wells (Flow) in complete medium. Each well contained 5×10^3 cells in the presence of $\log_2$ dilutions of TCGF supernatants. After 20 h in culture at 37°C in 5% CO_2 in air, wells were pulsed for 4 h with 0.5 μCi of tritiated thymidine (^{3}H-TdR, New England Nuclear, Boston, MA), harvested on glass fiber strips, and ^{3}H-TdR uptake measured in a liquid scintillation counter. A 50% endpoint for uptake of ^{3}H-TdR was designated as 1 U of TCGF activity (41).

Immune Responses in B Cell Cultures. C3H/HeN mice were given 0.075 ml of rabbit anti-thymocyte sera (Microbiological Associates, Walkersville, MD) 48 h before sacrifice. Splenic or PP single cell suspensions were prepared (36) and treated with monoclonal anti-T cell cocktail as described above. The cocktail of monoclonal antibodies was added to the cell pellet in medium containing 2% FCS (30 min/4°C) followed by addition of anti-rat IgG and C (30 min, 37°C). Cells were washed twice in minimal essential medium (Gibco) supplemented with L-glutamine, gentamicin, sodium bicarbonate, sodium pyruvate, nonessential amino acids (incomplete MEM), and resuspended in complete MEM medium (incomplete MEM plus 10% FCS and 2-ME) (35, 36). This population of B cells was free of residual T cells since <1% of cells stained with FITC-anti–Thy-1.2, and did not elicit mitogenic responses to PHA or Con A or form PFC responses to several thymic-dependent antigens. Appropriate concentrations of B cells and cloned T cells were added to macroculture or microculture (Linbro) wells containing erythrocyte antigen and incubated at 37°C in an atmosphere of 7% O_2, 10% CO_2, and 83% N_2.

Depletion/Enrichment of sIgA$^+$ B cells by Flow Cytometry. For depletion of sIgA$^+$ B cells, either splenic or PP B cells were separated on Ficoll-hypaque gradients, washed, and cells $(2-6 \times 10^7)$ were stained with 50 μl of fluorescein isothiocyanate–conjugated, affinity-purified goat IgG specific for mouse α heavy chain (FITC-anti-α) (Southern Biotechnology Co., Birmingham, AL) by incubation for 30 min at 4°C. Cells were then washed twice with incomplete MEM and resuspended to 6×10^6 cells/ml for cell sorting. Flow cytometry was performed using a FACS IV (Becton-Dickinson and Co., Sunnyvale, CA) equipped with a logarithmic amplifier. Aliquots of cells were then examined with a Leitz fluorescent microscope (E. Leitz, Inc., Rockleigh, NJ) for the presence of sIgA$^+$ B cells. Examination of 1,500 cells revealed the presence of less than 2 total sIgA$^+$ cells.

For collection of sIgA$^+$ B cells from PP cell cultures, FITC anti-α treated cell populations were sorted into sIgA$^+$ and sIgA$^-$ cell pools. Due to the low cell yields of sIgA$^+$ B cells, it was necessary to perform microculture in vitro assays. Accessory cells were provided by addition of $1-2 \times 10^5$ irradiated (3,000 rads) C3H/HeN splenic mononuclear cells to each well and incubation for 2 h at 37°C in 5% CO_2, followed by the removal of nonadherent cells before addition of sorted cells $(1 \times 10^5$ cells/well) and T cells $(1 \times 10^3$ cells) for culture.

Separation of sIgA$^+$ and sIgA$^-$ B Cells by the "Panning" Method. Enriched sIgA$^+$ and sIgA$^-$ B cell populations were prepared from PP B cell preparations (described above) by pretreating the B cells with goat IgG anti-mouse α heavy chain, followed by adherence to rabbit anti-goat IgG-coated plates (42). Affinity-purified rabbit anti-goat IgG antibody was absorbed with mouse immunoglobulin before coating of the petri dishes. For panning, rabbit anti-goat IgG antibody (10 ml at 25 μg/ml in 0.05 M Tris buffer, pH 9.5) was added to petri plates (15 $\times$ 100 mm, Falcon Labware, Oxnard, CA) and plates were incubated at room temperature for 40 min. Plates were washed four times with phosphate-buffered saline (PBS) and finally with PBS containing 1% FCS. Goat IgG anti-mouse α-

treated PP B cells in 3 ml of PBS containing 5% FCS were poured onto these precoated plastic plates. The dishes were incubated at 4°C for 70 min. Nonadherent cells were harvested with four gentle washes using PBS containing 1% FCS. In some experiments, pooled nonadherent cells were further treated with goat anti-mouse α (Meloy Laboratories, Springfield, VA) and complement. These preparations served as sIgA⁻ B cell cultures. Adherent cells were recovered by addition of PBS containing 1% FCS and by vigorous pipetting of media over the plate surface. Recovered cells were further treated with goat anti-mouse μ and γ (Meloy) and complement before their use as sIgA⁺ B cell cultures. Aliquots of cells were then examined with a Leitz fluorescent microscope for the presence of sIgA⁺ cells as described above, <5 sIgA⁺ B cells were seen per 1,500 cells examined in the nonadherent preparation, while >95% of the adherent cell population was sIgA⁺. Either sIgA⁺ or sIgA⁻ B cells (4 × 10⁵ cells/well) were cultured with cloned PP Th A cells (4 × 10³/well), accessory cells (described above), and SRBC antigen in microculture plates.

Splenic B Cell Cultures from Young Mice.   Single cell suspensions of spleen were obtained from C3H/HeN mice of various ages (days 1, 4, 7, 14, 17, 21, 25, 28, 31, 42, and 49). B cell cultures were prepared using monoclonal anti–T cell cocktail antibodies and rabbit C as described above. B cells were added (2.5 × 10⁶/well) to macroculture wells containing SRBC (1–2 × 10⁶) and appropriate cloned T cells (1 × 10⁴/well) and cultured as described above.

PFC Assay.   After 5 d of culture, nonadherent cells were removed from macroculture wells, washed in Hanks' balanced salt solution (HBSS; Gibco), and resuspended in HBSS at appropriate dilutions for plaque assay. Cultures were assesed for direct (IgM) and indirect (IgG₁, IgG₂, and IgA) anti-SRBC PFC responses as previously described (35, 36). In addition, IgG₃ anti-SRBC PFC responses were determined using an optimal concentration of goat anti-mouse γ_3 antisera (Litton Bionetics). For microplate bioassay, triplicate cultures were assessed on day 5 for either IgM or IgA anti-SRBC PFC by the Cunningham-Szenberg modification of the hemolytic plaque technique (43).

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

Results

PP Th A Cells Collaborate with Surface IgA Bearing B Cells from Peyer's Patches for In Vitro IgA Responses.   PP Th A clones have been propagated in culture for ~2 years and the cells have maintained their ability to provide preferential IgA anti-SRBC PFC responses in B cell cultures. We have maintained four clones continuously, two (PP Th A #1 and #18) that promote some IgM and high IgA, and two (PP Th A #9 and #19) that support low, but significant IgM, IgG₁, IgG₂, IgG₃, and high IgA anti-SRBC PFC responses (reference 36, and data not shown). For convenience, we present data only for PP Th A #1 and #9 in this paper. Other experiments indicate that clones #18 and #19 exhibit identical properties to those reported here.

Previously we have shown that PP Th A cells support comparatively higher IgA anti-SRBC PFC responses in PP, than with splenic, B cell cultures (36). In this regard, PP contain a higher frequency of sIgA⁺ B cells, and the simplest explanation for higher IgA isotype responses with cloned PP Th A cells would be that the Th cell collaborates selectively with postswitched (sIgA⁺) B cells for isotype-specific responses. To test this, PP B cells were separated into sIgA⁻ and sIgA⁺ populations before culture with cloned PP Th A cells and SRBC.

In the first experiments, PP B cells were treated with FITC-anti-α antibodies and sorted into sIgA⁺ and sIgA⁻ subpopulations by automated flow cytometry.

When sIgA⁻ B cells were incubated with PP Th A cells and antigen, the IgA response was greatly reduced in comparison with the nonsorted B cell population (Table I). In contrast, sIgA⁺ B cells and PP Th A cells gave an elevated IgA response to SRBC. The altered response pattern was isotype-specific since sIgA⁻ B cells and unsorted PP B cells gave similar IgM responses, while sIgA⁺ B cell subpopulations supported high IgA, but low IgM, PFC responses. These results suggest that PP Th A cells preferentially collaborate with committed sIgA⁺ B cells for IgA isotype responses and that IgM responses are induced in the sIgA⁻ B cell population.

This premise was further tested using a second method for B cell separation according to surface Ig isotype; PP B cells were separated into nonadherent (sIgA⁻) and adherent (sIgA⁺) populations by panning. Again, PP Th A cells supported normal IgM and low IgA responses in the sIgA⁻ B cell culture, whereas the enriched sIgA⁺ (>95%) cultures with PP Th A cells and SRBC gave few IgM and elevated IgA PFC responses (Table II). Thus, two separate methods for separation of B cell subpopulations gave essentially identical results indicating that the cloned PP Th A cells preferentially collaborate with mature B cells for isotype-specific responses.

Evidence that PP Th A Cells Collaborate with Splenic B Cells Committed to IgA. Our previous studies showed that vigorous IgA anti-SRBC responses were obtained in splenic B cell cultures with cloned PP Th A cells (36). In the present experiments, splenic B cells were treated with FITC-anti-α and sIgA⁺ cells were depleted by FACS. This treatment significantly reduced IgA anti-SRBC PFC responses, but did not alter the IgM response induced with PP Th A cells and antigen (Table III). Since this treatment did not significantly alter the IgM response pattern, the results again suggest that PP Th A cells collaborate with B cells expressing surface IgA for IgA responses (Table III).

TABLE I

PP Th A Cell Help in sIgA⁺ and sIgA⁻ Peyer's Patch B Cell Cultures Separated by Flow Cytometry*

PP Th A cells added to B cell cultures (clone no.)	B cell preparation	Anti-SRBC PFC/culture‡	
		IgM	IgA
1	Total B cells	62 ± 2	182 ± 7
	sIgA⁻ B cells	57 ± 8	28 ± 2
	sIgA⁺ B cells	11 ± 1	308 ± 65
9	Total B cells	30 ± 3	97 ± 13
	sIgA⁻ B cells	27 ± 2	17 ± 2
	sIgA⁺ B cells	8 ± 2	234 ± 84

* Peyer's patch B cell preparations were incubated with FITC-labeled anti-α antibody and cells sorted by FACS into sIgA⁻ and sIgA⁺ populations. B cells were cultured (1 × 10⁵ cells/well) with the appropriate PP Th A clone (1 × 10³ cells) and SRBC (5 × 10⁴). IgM and IgA anti-SRBC responses were assessed on day 5 of culture.

‡ Values are the mean anti-SRBC PFC responses per culture ± SEM from duplicate cultures per experiment and three separate experiments. B cell cultures immunized with SRBC did not support anti-SRBC PFC responses. Nonimmunized control cultures gave less than 2 anti-SRBC PFC/culture.

TABLE II

PP Th A Cell Help in sIgA⁺ and sIgA⁻ Peyer's Patch B Cell Cultures
Separated by Panning*

PP Th A cells added to B cell cultures (clone no.)	B cell preparation	Anti-SRBC PFC/Culture‡	
		IgM	IgA
1	Total B cells	203 ± 27	671 ± 71
	sIgA⁻ B cells	233 ± 16	131 ± 15
	sIgA⁺ B cells	63 ± 4	1,173 ± 94
9	Total B cells	113 ± 14	372 ± 28
	sIgA⁻ B cells	102 ± 6	92 ± 13
	sIgA⁺ B cells	41 ± 5	987 ± 76

* Peyer's patch B cell preparations were treated with goat IgG anti-mouse α antibody and cells were incubated in rabbit anti-goat IgG coated petri dishes. Panned B cells (sIgA⁻ or sIgA⁺) were cultured (4 × 10⁵ cells) with the PP Th A cells (4 × 10⁵) and SRBC (2 × 10⁵). IgM and IgA anti-SRBC PFC responses were assessed on day 5 of culture.

‡ Values are the mean anti-SRBC PFC responses per culture ± SEM from duplicate cultures per experiment and two separate experiments. B cell cultures immunized with SRBC did not support anti-SRBC PFC responses. Nonimmunized control cultures gave less than 3 anti-SRBC PFC/culture.

TABLE III

Evidence That PP Th A Cells Provide Help for Splenic sIgA⁺ B Cells for IgA
Responses*

PP Th A cells added to B cell cultures (clone no.)	B cell preparation	Anti-SRBC PFC/culture‡	
		IgM	IgA
1	Total B cells	304 ± 15	736 ± 17
	sIgA⁻ B cells (FACS)	291 ± 21	162 ± 19
9	Total B cells	213 ± 16	553 ± 32
	sIgA⁻ B cells (FACS)	206 ± 18	119 ± 14

* Splenic B cells were incubated with FITC-labeled anti-α antibody and sIgA⁺ cells removed by FACS. B cells were cultured (1 × 10⁶ cells/well) with the appropriate cloned PP Th A cells (1 × 10⁴ cells) and SRBC (5 × 10⁵). IgM and IgA anti-SRBC PFC responses were assessed on day 5 of culture.

‡ Values are the mean anti-SRBC PFC responses per culture ± SEM from triplicate cultures per experiment and four separate experiments. B cell cultures immunized with SRBC gave <5 anti-SRBC PFC/culture. Nonimmunized control cultures gave <9 anti-SRBC PFC/culture.

The finding that both splenic and PP B cell populations depleted of sIgA⁺ cells by either flow cytometry or panning give rise to limited numbers of IgA PFC suggests the PP Th A cells either (a) induce a switch step or (b) support IgA responses in B cell subpopulations with insufficient sIgA for their separation. In the latter case, B cells may appear in an earlier stage of differentiation, but are nevertheless committed to IgA expression. To examine this point more closely, splenic B cell cultures were incubated with PP Th A cells (clone #1 or #9) and

SRBC for 2 d in culture. Nonadherent cells were removed, T cells depleted with anti–T cell antibodies and rabbit C, and $sIgA^+$ B cells removed by FACS and $sIgA^-$ B cells recultured with PP Th A cells and antigen. The data presented in Table IV indicate that $sIgA^-$ B cell cultures gave rise to low IgA and normal IgM anti-SRBC PFC responses (Table IV). PP Th A clone #9 also supported low IgG_1, IgG_2, and IgG_3 anti-SRBC PFC responses in both normal and $sIgA^-$ B cell cultures (data not shown). These results support the conclusion that PP Th A cells preferentially help in the induction of IgA plasma cell differentiation by collaboration with precursor cells which express sIgA.

Ontogeny of B Cells Helped by PP Th A Clones. It is well established that young mice have an immature B cell population, and more mature B cells ($sIgM^+$, $sIgD^+$, and those committed to other isotypes) accumulate over the first few weeks of life (44–46). It was therefore of interest to examine the ability of PP Th A cells to support anti-SRBC responses in splenic B cell cultures derived from mice of varying ages as shown in Fig. 1. Both PP Th A #1 and #9 support low but signfiicant IgM responses by 14 d of age. Interestingly, clone #9 supports IgG_1, IgG_2, and IgG_3 responses beginning at 3 wk of age. Neither clone supported IgA anti-SRBC responses until ~28 d of age, and maximal IgA responses were only seen in splenic B cell cultures derived from young adult mice (Fig. 1). These results indicate that relatively mature IgA B cells are the targets of PP Th A cells and under the influence of the latter are directed to final differentiation with IgA synthesis and secretion.

TABLE IV

Removal of $sIgA^+$ B Cells from Ongoing Spleen Cell Cultures Abrogates PP Th A Help*

PP Th A cells added to B cell cultures (clone no.)	B cell preparation	Anti-SRBC PFC/culture[‡]	
		IgM	IgA
1	Total B cells (48-h culture)	219 ± 13	471 ± 22
	$sIgA^-$ B cells (48-h culture)	192 ± 11	98 ± 9
9	Total B cells (48-h culture)	98 ± 4	251 ± 17
	$sIgA^-$ B cells (48-h culture)	101 ± 5	63 ± 8

* Splenic B cells (2.5×10^6 cells/well) were incubated with cloned PP Th A cells (2×10^4) and SRBC ($1-2 \times 10^6$) for 48 h, and nonadherent cells removed. T cells were depleted with anti–Thy-1.2 and C, B cells were mixed with FITC-labeled anti-α, and $sIgA^+$ cells were removed by FACS. Cells were recultured (5×10^5/well) with appropriate cloned PP Th A cells (5×10^5) and SRBC ($2-3 \times 10^5$). IgM and IgA anti-SRBC PFC responses were assessed on day 5 of culture.

‡ Values are the mean anti-SRBC PFC responses per culture ± SEM from triplicate cultures per experiment and two separate experiments. B cell cultures immunized with SRBC gave 0–3 anti-SRBC PFC/culture. Nonimmunized controls gave less than 6 anti-SRBC PFC/culture.
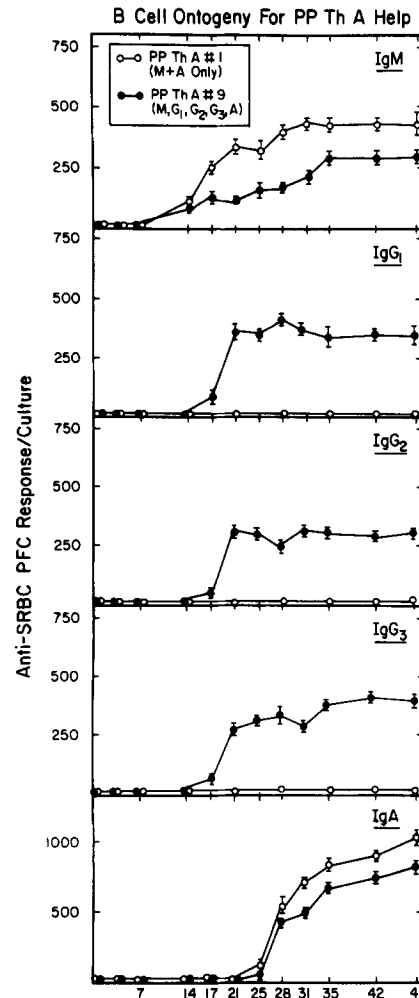
B Cell Ontogeny For PP Th A Help

IgM

o—o PP Th A # 1
(M+A Only)

●—● PP Th A # 9
(M,G₁,G₂G₃,A)

IgG₁

IgG₂

IgG₃

IgA

Anti-SRBC PFC Response/Culture

7   14 17 21 25 28 31 35   42   49

FIGURE 1.   B cell maturational requirements for PP Th A isotype-specific help. PP Th A #1 (-O-) or #9 (-●-) cells were added (2 × 10⁴) to splenic B cell cultures (2.5 × 10⁶). The isotype (IgM, IgG₁, IgG₂, IgG₃ and IgA) of the anti-SRBC PFC response was determined on day 5 of culture. Values are expressed as the mean PFC response/culture ± SEM from triplicate cultures/experiment and two separate experiments. Control (B cells incubated with SRBC) responses ranged from 0–12 anti-SRBC PFC/culture. Nonimmunized control cultures gave less than 15 anti-SRBC PFC/culture.

Discussion

B cells expressing surface IgM may switch to express other isotypes, and two opposing views of this heavy chain isotype switching have been presented. In the first model, it was concluded that B cells undergo early intraclonal switching from IgM to the expression of other isotypes independently of antigen and T cell influences; the latter were envisioned as clonal selective forces governing growth and terminal differentiation of precommitted B cells (47). In keeping with this idea, class-specific T cells may recognize surface Ig on committed B cells and select from this population for isotype-specific help (28, 32, 34).

Alternatively, heavy chain isotype switches have been considered to be antigen-induced events (48, 49) directed by helper T cells (38, 39, 50–52) or their soluble factors (53). The two hypotheses imply very different mechanisms by which T cells may influence the immunoglobulin isotype produced in an antibody response. For T cells to preferentially enhance differentiation of isotype-precommitted B cells, the T cell would presumably require an isotype-specific receptor. On the other hand, for T cells or their soluble factors to induce an $sIgM^+$ B cell to switch to the expression of another isotype, a mechanism for inducing selective C_H gene expression, probably by rearrangement, would appear to be necessary.

In the present studies we have used clones of antigen-specific T helper cells to examine this issue. The results suggest that PP Th A cells can recognize and provide help for B cells already committed to IgA expression. First, depletion of either splenic or PP B cell cultures of $sIgA^+$ cells markedly diminish PP Th A supported IgA anti-SRBC responses. Conversely, enrichment of $sIgA^+$ B cells from PP, either by fluorescence-activated cell sorting or panning with anti-α antibodies, greatly enhances IgA anti-SRBC responses in cultures containing $sIgA^+$ B cells and PP Th A cells. The latter result provides direct evidence that isotype-specific responses are dependent upon IgA-committed B cells and isotype-specific T cells, since cultures contained only antigen, cloned PP Th A helper cells and $sIgA^+$ B cells (>95% purity).

It should be noted that in addition to high IgA responses, the individual PP Th A clones supported either limited IgM or low IgM, IgG_1, IgG_2, and IgG_3 anti-SRBC PFC responses. Depletion of $sIgA^+$ B cells from either splenic or PP B cell cultures did not alter IgM responses and, with the second PP Th A clone group, IgG_1, IgG_2, and IgG_3 anti-SRBC PFC responses. This result suggests that the limited help provided by the PP Th A cells for IgM and IgG precursors is independent of the help delivered to $sIgA^+$ precursors. Our failure to detect significant IgA responses in $sIgA^-$ B cell cultures also suggests that these clones of PP Th A cells do not direct class switching to IgA.

Although splenic B cell populations contain a relatively low percentage of $sIgA^+$ B cells (46, 54), vigorous IgA responses were elicited in the presence of PP Th A cells. This result could suggest that the T cells directed switching of B cells or that the spleen contains B cells in different stages of isotype expression, including those committed to IgA, but which express insufficient surface IgA for their detection. We observed, however, that removal of $sIgA^+$ B cells from spleen significantly depleted IgA responses (Table III). Moreover, incubation of PP Th A cells with splenic B cells and antigen for 48 h, followed by removal of $sIgA^+$ B cells, also reduced IgA responses (Table IV), but did not alter the IgM response pattern. If switches of $sIgM^+$ B cells to $sIgA^+$ B cells were frequent occurrences in SRBC-responsive clones, we would expect the precursor $sIgM^+$ B cells to be diminished in a 48-h culture. To the contrary, removal of $sIgA^+$ B cells from spleen or PP cultures was without effect on IgM responses and only reduced the IgA response to the antigen.

Our studies with splenic B cells from young mice indicate that PP Th A cells act on a relatively mature subpopulation of B cells. B cells bearing IgG or IgA isotypes are generated over the first few weeks of life (46). Our studies with PP Th A clone #9 and sheep erythrocyte-specific B cells from mice of different ages

demonstrate that the inducible IgM precursors precede the development of IgG and IgA precursors. If T cell switch influences were required for commitment to IgA, one would expect IgA responses in cultures of spleen cells from newborn mice. On the contrary, the IgA-responsive cells were the last to develop, and a normal IgA response was not seen until the donor mice reached the age of 7 wk. These results suggest that the isotype-specific T helper cells collaborate with a mature, surface IgA-bearing population of B cells for the induction of IgA responses.

Cultures of newborn mouse liver containing μ^+ pre-B cells and sIgM$^+$ B cells gave rise to IgG (γ1, γ2a, γ2b, and γ3 subclass representation) and IgA B cells (55, 56). Isotype switching by the immature B cells occurred during the first 4 d of culture, and the switch frequency was normal in cultures of liver cells from athymic nu/nu newborns (56). These results support the hypothesis that T cells are not essential for the initiation of isotype switching. Our present results would suggest that immature IgA B cells later acquire the capacity to respond to help provided by PP Th A cells. Preferential T cell help for B cells committed to the expression of the different IgG subclasses has also been shown in human (57, 58) and in mouse model systems (59).

The PP Th A clones used in the present studies have been shown to express Fcα receptors (36). Others have provided evidence indicating that a subpopulation of human T cells bearing Fcα receptors can preferentially enhance IgA synthesis in PWM-stimulated cultures (60). Human T cell hybridomas have been described that produce soluble factors capable of selectively enhancing differentiation of IgA-bearing leukemia cells and tonsillar B cell precursors of IgA-secreting cells (37). A possible mechanism by which PP Th A cells could recognize and help IgA-bearing B cells is via the production of Fcα receptors. A precedent for such a mechanism is the demonstration of soluble IgE-binding factors that can either enhance or suppress IgE responses depending upon the degree of glycosylation (32). Moreover, in recent studies, we have obtained evidence indicating that monoclonal IgA molecules can selectively inhibit the IgA response promoted by our PP Th A clones (manuscript in preparation).

Finally, it should be mentioned that our studies do not preclude the possibility that T cells may induce isotype switching of B cells in some instances. Support for a switch T cell for IgA expression has come from studies of T cell clones derived from concanavalin A–stimulated murine PP. These cloned T cells appear to promote switching of sIgM$^+$ B cells to the expression of sIgA (38, 39). In addition, a T cell hybridoma and two T cell lines have been shown to produce lymphokines that can preferentially direct LPS-stimulated sIgG$^-$ B cells to differentiate into cells producing IgG$_1$ (53). Elucidation of the nature of these class-specific differentiation factors should help to resolve this issue.

Summary

The nature of the IgA B cell precursors that receive preferential help from selected clones of T helper cells from mouse Peyer's patches (PP Th A) were studied. Activation of the PP Th A clones required the presence of antigen, sheep erythrocytes (SRBC), in a culture system supporting development of antibody-secreting plasma cells. Two types of PP Th A cells were used. Both

gave vigorous IgA responses; the first also supported low IgM, and the second low IgM and IgG subclass antibody responses. Removal of sIgA⁺ B cells from either splenic or PP B cell cultures selectively depleted precursors of IgA antibody producers. Cultures of purified sIgA⁺ B cells, cloned PP Th A cells and SRBC, selectively yielded IgA antibody producers. Finally, PP Th A cells did not support IgA responses in B cell cultures derived from spleens of young mice (days 1–25), and full IgA responses were not seen until the donor mice were 6–7 wk of age. These results suggest that cloned T helper cells can recognize and collaborate with mature, IgA committed B cells.

References

1. Mitchison, N. A. 1967. Antigen recognition responsible for the induction *in vitro* of the secondary response. *Cold Spring Harbor Symp. Quant. Biol.* 31:431.
2. Rajewsky, K., and E. Rottlander. 1967. Tolerance specificity and the immune response to lactic dehydrogenase isoenzymes. *Cold Spring Harbor Symp. Quant. Biol.* 32:547.
3. Mitchison, N. A. 1971. The carrier effect in the secondary response to hapten-protein conjugates. II. Cellular cooperation. *Eur. J. Immunol.* 1:18.
4. Katz, D. H., and B. Benacerraf. 1972. The regulatory influence of activated T cells to B cell responses to antigen. *Adv. Immunol.* 15:1.
5. Janeway, C. A., Jr. 1975. Cellular cooperation during *in vivo* anti-hapten antibody responses. I. The effect of cell number on the response. *J. Immunol.* 114:1394.
6. Rosenthal, A. S., and E. M. Shevach. 1973. Function of macrophages in antigen recognition by guinea pig T lymphocytes. I. Requirement for histocompatible macrophages and lymphocytes. *J. Exp. Med.* 138:1194.
7. Erb, P., and M. Feldmann. 1975. The role of macrophages in the generation of T-helper cells. II. The genetic control of the macrophage-T cell interaction for helper cell induction with soluble antigens. *J. Exp. Med.* 142:460.
8. Pierce, C. W., J. A. Kapp, and B. Benacerraf. 1976. Regulation by the H-2 gene complex of macrophage-lymphoid cell interactions in secondary antibody responses in vitro. *J. Exp. Med.* 144:371.
9. Katz, D. H., T. Hamoaka, and B. Benacerraf. 1973. Cell interactions between histoincompatible T and B lymphocytes. II. Failure of physiologic cooperative interactions between T and B lymphocytes from allogeneic donor strains in humoral response to hapten-protein conjugates. *J. Exp. Med.* 137:1405.
10. Sprent, J. 1978. Restricted helper function of F₁ hybrid T cells positively selected to heterologous erythrocytes in irradiated parental strain mice. I. Failure to collaborate with B cells of the opposite parental strain not associated with active suppression. *J. Exp. Med.* 147:1142.
11. Sprent, J. 1978. Restricted helper function of F₁ hybrid T cells positively selected to heterologous erythrocytes in irradiated parental strain mice. II. Evidence for restrictions affecting helper cell induction and T-B collaboration, both mapping to the K-end of the H-2 complex. *J. Exp. Med.* 147:1159.

12. McDougal, J. S., and S. P. Cort. 1978. Generation of T helper cells *in vitro*. IV. F_1 T helper cells primed with antigen-pulsed parental macrophages are genetically restricted in their antigen-specific helper activity. *J. Immunol.* 120:445.

13. Swierkosz, J. E., K. Rock, P. Marrack, and J. W. Kappler. 1978. The role of H-2 linked genes in helper T-cell function. II. Isolation on antigen-pulsed macrophages of two separate populations of F_1 helper T cells each specific for antigen and one set of parental H-2 products. *J. Exp. Med.* 147:554.

14. Erb, P., B. Meier, T. Matsunaga, and M. Feldmann. 1979. Nature of T-cell macrophage interaction in helper-cell induction in vitro. II. Two stages of T-helper-cell differentiation analyzed in irradiation and allophenic chimeras. *J. Exp. Med.* 149:686.

15. Singer, A., K. S. Hathcock, and R. J. Hodes. 1980. Cellular and genetic control of antibody responses. VIII. MHC restricted recognition of accessory cells, not B cells, by parent-specific subpopulations of normal F_1 T helper cells. *J. Immunol.* 124:1079.

16. Shih, W. W. H., P. C. Matzinger, S. L. Swain, and R. W. Dutton. 1980. Analysis of histocompatibility requirements for proliferative and helper T cell activity. T cell populations depleted of alloreactive cells by negative selection. *J. Exp. Med.* 152:1311.

17. Watson, J. 1979. Continuous proliferation of murine antigen-specific helper T lymphocytes in culture. *J. Exp. Med.* 150:1510.

18. Jones, B., and C. A. Janeway, Jr. 1981. Cooperative interaction of B lymphocytes with antigen-specific helper T lymphocytes is MHC restricted. *Nature (Lond.).* 292:547.

19. Cammisuli, S., and M. H. Schreier. 1981. Individual clones of carrier-specific T cells help idiotypically and isotypically heterogeneous anti-hapten B cell-responses. *Immunology.* 43:581.

20. Andersson, J., M. H. Schreier, and F. Melchers. 1980. T-cell–dependent B-cell stimulation is H-2 restricted and antigen dependent only at the resting B-cell level. *Proc. Natl. Acad. Sci. USA.* 77:1612.

21. Andersson, J., and F. Melchers. 1981. T cell-dependent activation of resting B cells: requirement for both non-specific unrestricted and antigen-specific Ia-restricted soluble factors. *Proc. Natl. Acad. Sci. USA.* 78:2497.

22. Asano, Y., M. Shigeta, C. G. Fathman, A. Singer, and R. J. Hodes. 1982. Role of the major histocompatibility complex in T cell activation of B cell subpopulations: a single monoclonal T helper cell population activates different B cell subpopulations by distinct pathways. *J. Exp. Med.* 156:350.

23. Singer, A., Y. Asano, M. Shigeta, K. S. Hathcock, A. Ahmed, C. G. Fathman, and R. J. Hodes. 1982. Distinct B cell subpopulations differ in their genetic requirements for activation by T helper cells. *Immunol. Rev.* 64:137.

24. Janeway, C. A., Jr., R. A. Murgita, F. I. Weinbaum, R. Asofsky, and H. Wigzell. 1977. Evidence for an immunoglobulin-dependent antigen-specific helper T cell. *Proc. Natl. Acad. Sci. USA.* 74:4582.

25. Bottomly, K., C. A. Janeway, Jr., B. J. Mathieson, and D. E. Mosier. 1980. Absence of an antigen-specific helper T cell required for the expression of the T15 idiotype in mice treated with anti-μ antibody. *Eur. J. Immunol.* 10:159.

26. Janeway, C. A. 1981. Idiotypic control: the expression of idiotypes and its regulation. *In* Strategies of Immune Regulation. E. E. Sercarz, and A. J. Cunningham, editors. Academic Press, New York. p. 179.

27. Nutt, N., J. Haber, and H. H. Wortis. 1981. Influence of *Igh*-linked gene products on the generation of T helper cells in the response to sheep erythrocytes. *J. Exp. Med.* 153:1225.

28. L'Age-Stehr, J. 1980. Priming of T helper cells by antigen-activated B cells: B cell-

primed Lyt-1$^+$ helper cells are restricted to cooperate with B cells expressing the *Igvh* phenotype of the priming B cells. *J. Exp. Med.* 153:1236.

29. Gleason, K., S. Pierce, and H. Köhler. 1981. Generation of idiotype-specific T cell help through network pertubation. *J. Exp. Med.* 153:924.

30. Gleason, K., and H. Köhler. 1982. Regulatory idotypes. T helper cells recognize a shared V$_H$ idiotope on phosphorylcholine-specific antibodies. *J. Exp. Med.* 156:539.

31. Herzenberg, L. A., K. Okumura, H. Cantor, V. L. Sato, F. W. Shen, E. A. Boyse, and L. A. Herzenberg. 1976. T-cell regulation of antibody responses: demonstration of allotype-specific helper T cells and their specific removal by suppressor T cells. *J. Exp. Med.* 144:330.

32. Ishizaka, K., J. Yodoi, M. Suemura, and M. Hirashima. 1983. Isotype-specific regulation of the IgE response by IgE-binding factors. *Immunology Today.* 4:192.

33. Elson, C. O., J. A. Heck, and W. Strober. 1979. T-cell regulation of murine IgA synthesis. *J. Exp. Med.* 149:632.

34. Rosenberg, Y. J., and J. M. Chiller. 1979. Ability of antigen-specific helper cells to effect a class-restricted increase in total Ig-secreting cells in spleen after immunization with the antigen. *J. Exp. Med.* 150:517.

35. Kiyono, H., J. R. McGhee, M. J. Wannemuehler, M. V. Frangakis, D. M. Spalding, S. M. Michalek, and W. J. Koopman. 1982. *In vitro* immune responses to a T-cell dependent antigen by cultures of disassociated murine Peyer's patch. *Proc. Natl. Acad. Sci. USA.* 79:596.

36. Kiyono, H., J. R. McGhee, L. M. Mosteller, J. H. Eldridge, W. J. Koopman, J. F. Kearney, and S. M. Michalek. 1982. Murine Peyer's patch T cell clones. Characterization of antigen-specific helper T cells for immunoglobulin A responses. *J. Exp. Med.* 156:1115.

37. Mayer, L., S. M. Fu, and H. G. Kunkel. 1982. Human T cell hybridomas secreting factors for IgA-specific help, polyclonal B cell activation, and B cell proliferation. *J. Exp. Med.* 156:1860.

38. Kawanishi, H., L. E. Saltzman, and W. Strober. 1983. Mechanisms regulating IgA class-specific immunoglobulin production in murine gut-associated lymphoid tissues. I. T cells derived from Peyer's patches that switch sIgM B cells to sIgA B cells in vitro. *J. Exp. Med.* 157:433.

39. Kawanishi, H., L. Saltzman, and W. Strober. 1983. Mechanisms regulating IgA class-specific immunoglobulin production in murine gut-associated lymphoid tissues. II. Terminal differentiation of postswitch sIgA-bearing Peyer's patch B cells. *J. Exp. Med.* 158:649

40. Farrar, J. J., J. Fuller-Farrar, P. L. Simon, M. L. Hilfiker, B. M. Stadler, and W. L. Farrar. 1980. Thymoma production of T cell growth factor (interleukin 2). *J. Immunol.* 125:2555.

41. Gillis, S., and J. Watson. 1981. Interleukin-2 dependent culture of cytotoxic T cell lines. *Immunological Rev.* 54:81.

42. Wysocki, L. J., and V. L. Sato. 1978. "Panning" for lymphocytes: a method for cell selection. *Proc. Natl. Acad. Sci. USA.* 75:2844

43. Cunningham, A. J., and A. Szenberg. 1968. Further improvements in the plaque technique for detecting single antibody-forming cells. *Immunology.* 14:599.

44. Abney, E. R., and R. M. E. Parkhouse. 1974. Candidate for immunoglobulin D present on murine B lymphocytes. *Nature (Lond.).* 252:600.

45. Vitetta, E. S., U. Melcher, M. McWilliams, M. E. Lamm, J. M. Phillips-Quagliata, and J. W. Uhr. 1975. Cell surface immunoglobulin. XI. The appearance of an IgD-like molecule on murine lymphoid cells during ontogeny. *J. Exp. Med.* 141:206.

46. Abney, E. R., M. D. Cooper, J. F. Kearney, A. R. Lawton, and R. M. E. Parkhouse.

1978. Sequential expression of immunoglobulin on developing mouse B lymphocytes: a systematic survey that suggests a model for the generation of immunoglobulin isotype diversity. *J. Immunol.* 120:2041.

47. Cooper, M. D., A. R. Lawton, and P. W. Kincade. 1972. A two-stage model for development of antibody-producing cells. *Clin. Exp. Immunol.* 11:143.

48. Pierce, C. W., S. M. Solliday, and R. Asofsky. 1972. Immune responses in vitro. IV. Suppression of primary γM, γG and γA plaque-forming cell responses in mouse spleen cell cultures by class-specific antibody to mouse immunoglobulins. *J. Exp. Med.* 135:675.

49. Gearhart, P. J., J. L. Hurwitz, and J. J. Cebra. 1980. Successive switching of antibody isotypes expressed within the lines of a B- cell clone. *Proc. Natl. Acad. Sci. USA.* 77:5424.

50. Pierce, S. K., and N. R. Klinman. 1975. The allogeneic bisection of carrier-specific enhancement of monoclonal B-cell responses. *J. Exp. Med.* 142:1165.

51. Mongini, P. K. A., W. E. Paul, and E. S. Metcalf. 1982. T cell regulation of immunoglobulin class expression in the antibody response to trinitrophenyl-Ficoll: evidence for T cell enhancement of immunoglobulin class switch. *J. Exp. Med.* 155:884.

52. Mongini, P. K. A., W. E. Paul, and E. S. Metcalf. 1983. IgG subclass, IgE, and IgA anti-trinitrophenyl antibody production within trinitrophenyl-Ficoll-responsive B cell clones: evidence in support of three distinct switching pathways. *J. Exp. Med.* 157:69.

53. Isakson, P. C., E. Puré, E. S. Vitetta, and P. H. Krammer. 1982. T cell-derived B cell differentiation factor(s): effect on the isotype switch of murine B cells. *J. Exp. Med.* 155:734.

54. Lawton, A. R., R. Asofsky, M. B. Hylton, and M. D. Cooper. 1972. Suppression of immunoglobulin class synthesis in mice. I. Effects of treatment with antibody to μ chain. *J. Exp. Med.* 135:277.

55. Kearney, J. F., A. R. Lawton, and M. D. Cooper. 1977. Multiple immunoglobulin heavy chain expression by LPS stimulated murine B lymphocytes. *ICN-UCLA Symp. Mol. Cell. Biol.* 6:313.

56. Calvert, J. E., M. F. Kim, W. E. Gathings, and M. D. Cooper. 1983. Differentiation of B lineage cells from liver of neonatal mice: generation of immunoglobulin isotype diversity *in vitro. J. Immunol.* 131:1693.

57. Mayumi, M., T. Kuritani, H. Kubagawa, and M. D. Cooper. 1983. IgG subclass expression by human B lymphocytes and plasma cells: B lymphocytes precommitted to IgG subclass can be preferentially induced by polyclonal mitogens with T cell help. *J. Immunol.* 130:671.

58. Kuritani, T., and M. D. Cooper. 1982. Human B cell differentiation. I. Analysis of immunoglobulin heavy chain switching using monoclonal anti-immunoglobulin M, G, and A antibodies and pokeweed mitogen-induced plasma cell differentiation. *J. Exp. Med.* 155:839.

59. Forni, L., and A. Coutinho. 1982. The production of membrane or secretory forms of immunoglobulins is regulated by C-gene-specific signals. *Nature (Lond.).* 299:173.

60. Endoh, M., H. Sakai, Y. Nomoto, Y. Tomino, and H. Kaneshige. 1981. IgA-specific helper activity of Tα cells in human peripheral blood. *J. Immunol.* 127:2612.